COMPUTER APPLICATIONS IN BIOINORGANIC CHEMISTRY

Peter Michael May

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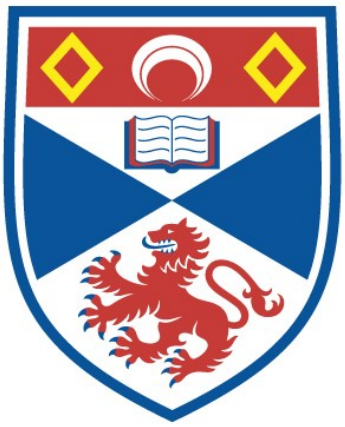
at the
University of St Andrews



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COMPUTER APPLICATIONS
IN
BIOINORGANIC CHEMISTRY

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by

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COMPUTER APPLICATIONS IN BIOINORGANIC CHEMISTRY

By Peter M. May

Nowadays, computers play an indispensible role in the determination of metal-ligand formation constants and in their application to various situations of analytical, industrial or biological interest. The development of programs and simulation techniques to meet some current problems in Bioinorganic chemistry constitutes the broad objective of the present research.

Consideration is given to the thermodynamic calculation of complex species concentrations in biological fluids. New methods of solving the mathematical relationships for metal-ligand solution equilibria, particularly in the simulation of large multicomponent systems, are investigated.

The ways in which computer simulations are involved in the determination of formation constants are discussed. Principles are developed and applied to problems concerning (i) the calibration of glass electrodes and (ii) the choice of complex species to describe metal-ligand systems under experimental investigation.

The function of transition elements in biological systems is briefly reviewed. Emphasis is given to the significance of low-molecular-weight complexes and how a knowledge of their *in vivo* behaviour can affect bioinorganic drug design. The relationship between copper and rheumatoid arthritis and the importance of equilibria in the regulation of iron metabolism are treated in some detail.

New simulation techniques are developed for blood plasma. The results successfully rationalise many bioinorganic phenomena. In particular, the relative ability of a series of chelating agents to compete with proteins for metal ions in plasma is correlated with the urinary excretion of trace elements that they cause. Further simulations extend the approach to other biofluids and to medical solutions intended for intravenous infusion.

DEDICATION

TO MY WIFE

QUOTATION

Models are undeniably beautiful, and man may justly be proud to be seen in their company. But they may have their hidden vices. The question is, after all, not only whether they are good to look at, but whether we can live happily with them.

A. Kaplan

"The Conduct of Enquiry"
Chandler, Intertext, 1964

PUBLICATIONS

The following publications have appeared whilst work for this thesis has been in progress.

P.M.May, P.W.Linder and D.R.Williams, Experientia 1976, 32 1492-4. "Ambivalent Fffect of Protein Binding on Computed Distributions of Metal Ions Complexed by Ligands in Blood Plasma".

P.M.May, P.W.Linder and D.R.Williams, J.Chem.Soc.Dalton Trans. 1977, 588-95. "Computer Simulation of Metal-ion Equilibria in Biofluids: Models for the Low-molecular-weight Complex Distribution of Calcium(II), Magnesium(II), Manganese(II), Iron(III), Copper(II), Zinc(II) and Lead(II) ions in Human Blood Plasma".

P.M.May and D.R.Williams, FEBS Letters 1977, 78, 134-9. "Computer Simulation of Chelation Therapy. Plasma Mobilizing Index as a Replacement for Effective Stability Constant".

P.M.May, J.Anatomy 1977, 123, 263. "Computer Models of Metal-ligand Equilibria in Biofluids and their Application to Cancer Chemotherapy".

P.M.May and D.R.Williams, Proc.Roy.Soc.Med.Supp.3, 1977, 70, 19-21. "Computer Models of Metal-ion Low-molecular-weight Equilibria in Plasma and the Influence of D-penicillamine, Triethylenetetramine and Ethylenediamine-tetraacetic acid".

G.E.Jackson, P.M.May and D.R.Williams, J.Inorg.Nucl.Chem. 1978, 40, 1189-94. "Metal-ligand Complexes Involved in Rheumatoid Arthritis. Part I. Justifications for Copper Administration".

M.Micheloni, P.M.May and D.R.Williams, J.Inorg.Nucl.Chem. 1978, 40, 1209-19. "Metal-ligand Complexes Involved in Rheumatoid Arthritis. Part IV. Formation Constant and Species Distribution Considerations for Copper(II)-Cystinate, -Oxidized Penicillaminate and -Oxidized Glutathionate Interactions and Considerations of the Action of Penicillamine *in vivo*".

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G.E.Jackson, P.M.May and D.R.Williams, FEBS Letters 1978, 90, 173-7. "The action of chelating agents in the removal of copper from ceruloplasmin - an *in vitro* study".

P.M.May and D.R.Williams, Nature, 1979, 281, 406. "Synergistic chelation therapy or mixed ligand complexes for plutonium and cadmium poisoning?".

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Mrs.P.Bevan for her superbly accurate typing.

CERTIFICATE

I hereby certify that PETER MICHAEL MAY has researched under my supervision and has fulfilled the conditions of Ordinance General Number 12 and Resolution of the St Andrews University Court 1967, number 1 and is qualified to submit this thesis in application for the degree of Doctor of Philosophy.

DAVID R. WILLIAMS

Department of Chemistry
UWIST, Cardiff

DECLARATION

I declare that this thesis is my own composition, that the work of which it is a record has been carried out by myself, and that it has not been submitted in any previous application for a higher degree.

The thesis describes the results of research performed in the Chemistry Departments of the University of St. Andrews and the University of Wales Institute of Science and Technology, Cardiff, under the supervision of Professor David R. Williams since January 1976.

PETER M. MAY

CONTENTS

Appendices

REFERENCE NUMBERING

The subject of this thesis covers a very broad literature. To deal with this, a computerised system for retrieving and citing references has been developed. It is based on a computer program called INDEX, the instructions and FORTRAN listing for which appear as Appendix A1.

In order to prevent duplication of the references cited in the text and those used in the computer system as well as to avoid transposition errors, the reference numbers used in this thesis have been taken directly from the computer database. Thus, they do not occur in the customary sequential manner. Those which have been specifically cited in the text have been marked in the list of references with an asterisk.

CHAPTER ONE

INTRODUCTION

1.1 Bioinorganic chemistry and computer applications

Bioinorganic chemistry is an interdisciplinary subject *par excellence*. Its span is particularly broad because it seeks to apply the disciplines of inorganic and physical chemistry to the whole range of biological sciences from biochemistry on the one hand to physiology and medicine on the other. Indeed, the past decade has seen it increasingly concerned with the balance between human health and disease. As in many interdisciplinary subjects, advances have often seemed easier to achieve because obvious directions for investigation have not previously been exploited. However, there has also been the corresponding disadvantage that a wide variety of material must be mastered in order to make progress. It is in this context that the computer has emerged as a powerful interdisciplinary tool: there are many ways it can bring together the theory, practices and data of different subject areas to help establish a new and self-consistent science.

Often the focus of a computer application will be a mathematical model. These are constructions which attempt to imitate reality by representing the various parts of a system and the interrelationships which exist between them. As such, they are an integral part of the scientific method, being used to make predictions from hypotheses so that the latter can be confirmed or rejected by further experiment [736]. Models, therefore, can only portray established data in a new way; they do not create knowledge. Yet, they are productive

because they uncover those unique aspects of the system which depend on the mutual interaction of its component parts.

Another less important but still valuable attribute of computers in interdisciplinary subject areas is their enormous capacity for storage and manipulation of data in its broadest sense. Their speed and reliability make it possible to accommodate systems that in the past would simply have been considered too complicated to analyse. Their robot-like character can also be exploited to provide many different kinds of scientific service which make the work of a researcher less tedious and more dependable. The role of computers in analytical chemistry, for example, has been reviewed by Childs *et al* [114,115]. On occasion, the physical magnitude of some tasks is such that a whole approach may be infeasible without computer aid. Thus it is both in terms of computer simulation models and computer service programs that the computer applications to bioinorganic chemistry are described in this work.

1.2 Previous work

One of the main themes of this thesis concerns computer simulation of the low-molecular-weight complexing equilibria in blood plasma as applied to a variety of situations having physiological or pharmaceutical interest. The work is founded on a model that was originally developed by the author in fulfilment of the requirements for the degree of Master of Science at the University of Cape Town (1976). Accordingly, much of the research described herein is an extension of the earlier project and certainly could not have been achieved without it. This dependence makes it necessary at the outset to demarcate between the present and the past contributions. Accordingly, the previous work may be briefly summarised as follows.

The initial model was designed to investigate the nature of the metal ion binding to ligands in normal, i.e. untreated, blood plasma. Perhaps the main objective was to accommodate the effects of metal protein binding on the computation of the complex species concentrations. This was accomplished in so far as the percentage distribution of transition metal ions amongst low-molecular-weight ligands was shown to be independent of the exact extent of metal protein binding. This is described more fully in Chapter 4. The collection, assembly and processing of the necessary data for the model was described. A computer program, ECCLES, was written to cope with the very large equilibrium systems that were simulated. Experimentally determined values for the

formation constants of the complexing reactions in the biofluid were collected from the literature. These were corrected whenever they were not applicable to physiological conditions of temperature and ionic strength. Where no experimental values were available, formation constants for complexes that seemed likely to be important were estimated from certain chemical trends. The results of the blood plasma model are summarised in Chapter 4. The work has been published as a thesis [736] and in two research papers [1234,1250].

Two of the service computer programs dealt with in this thesis, namely MIX and INDEX, were also originally written as part of the M.Sc research. Since then these programs, as well as ECCLES, have been considerably developed and/or extended, as outlined in Chapters 2 and 4. In the case of both program ECCLES and program INDEX, the FORTRAN coding had to be rewritten to make the programs compatible with the St Andrews' IBM 360/44 computer. Program MIX was considerably modified for the same reason.

1.3 Objectives and summary of the present research

The usual goal of those researching bioinorganic equilibria can be stated very simply: it is to measure the formation constants of biologically relevant metal complexes and to use this information to understand how the complex may be involved in the metabolism of the metal ion. For most purposes, the formation constant measurements are made using glass electrode potentiometry and are applied to the *in vivo* situation by some kind of simulation. This procedure requires a considerable amount of data processing and involves a variety of calculations. Certain elements of this work can only be performed by computer and many others prove very tedious to do manually. A number of computer programs have thus been produced by researchers in this field. However, several areas remain in which further computer applications would be very useful.

The development of computer programs and computer simulation techniques to meet some of these needs constitutes the broad objective of the present research. The more specific details can be summarised as follows.

The thermodynamic application of metal-ligand equilibrium constants to calculate complex species concentrations in biological fluids is considered. Formation constants for a large number of therapeutic chelating agents are critically selected from the literature and adjusted to suit biological conditions of temperature and ionic strength. New methods of solving the mathematical equations for metal-

ligand solution equilibria, particularly in the simulation of large multicomponent systems, are investigated. These efforts are applied to biological systems by modification of the ECCLES program and the extension of its database.

The ways in which computer simulations are involved in the determination of formation constants are outlined. The discussion focuses on the role of simulation in the optimisation of titration parameter values. Principles are developed and applied to problems concerning (i) the calibration of glass electrodes and (ii) the choice of complex species to describe metal-ligand systems under experimental investigation.

The function of transition metal ions in biological systems is briefly reviewed. Emphasis is placed on the biological significance of low-molecular-weight complexes and how a knowledge of their *in vivo* behaviour can affect bioinorganic drug design. Two areas are treated in detail. These are (i) the relationship between copper and rheumatoid arthritis and (ii) the biological transport of iron and the importance of equilibria in the regulation of its metabolism.

New computer simulation techniques are developed for blood plasma. By considering the *in vivo* complexing ability of chelating agents relative to one another, the difficulties associated with metal protein binding in models where exogenous drugs have been administered, are bypassed. The results successfully rationalise many bioinorganic phenomena that

hitherto have been incompletely understood. In particular, the relative ability of a series of medical chelating agents to compete with proteins for metal ions in plasma is correlated with the urinary excretion of trace elements that they cause. Further simulations extend this type of approach to other biofluids and to solutions intended for intravenous infusion.

CHAPTER TWO

COMPUTER SIMULATION OF METAL ION AND LIGAND EQUILIBRIA

2.1 The thermodynamics of metal ion and ligand interactions

Thermodynamics is a study of the effects of energy upon matter [639]. It is founded essentially upon three postulates, called the first, second and third laws, which summarise certain basic properties of energy that have been thoroughly established by cumulative scientific observation. Together these principles lead to a comprehensive and general description of how energy is liberated or consumed when chemical substances react. Thermodynamics can thus be used to predict relationships between various quantities involved in the reaction. Above all, it indicates whether a particular process is possible or not under a given set of conditions. This is achieved by reference to an equilibrium state, i.e. the final position of a chemical reaction which is defined as being invariant with time.

It is found by experiment that when substances are brought together in a closed system so as to undergo chemical reaction, the conversion of reactants to products always remains incomplete no matter how long the process is allowed to continue. Sooner or later the decreasing concentrations of reactants and the increasing concentrations of products level off and become constant. The state in which the concentrations no longer change is known as the state of chemical equilibrium.

Thermodynamics deals quantitatively with reactions at equilibrium. For this reason it is eminently applicable to one of the commonest problems in bioinorganic chemistry,

namely, how to determine the concentrations of complex species in biological systems. As will be discussed in greater detail under Section 4.1, many metal-ligand complexes of great physiological importance occur in amounts far below the present level of analytical detection. Thus, thermodynamic calculations provide the only available means for obtaining information of this kind..

It is necessary at this stage to consider briefly some of the strengths and weaknesses of a thermodynamic approach. As mentioned above, this is an experimentally based discipline. So, it does not depend on theories concerning the existence of atoms or molecules [639]; for example, the kinetic theory of matter, statistical mechanics and the Debye-Hückel theory lie outside its scope. As a consequence, thermodynamic results yield no direct information about atomic or molecular structure. They can likewise not be associated with reaction rates or mechanisms. It is in this restriction, however, that thermodynamics has its greatest strength. Since it makes no fundamental assumptions other than those postulated in the three laws its validity does not depend on anything else. It is no wonder Einstein commented as follows:

"A theory is the more impressive the greater is the simplicity of its premises, the more different are the kinds of things it relates, and the more extended is its area of applicability. Therefore, the deep impression which classical thermodynamics made upon me. It is the only physical theory

theory of universal content concerning which I am convinced that, within the framework of applicability of its basic concepts, will never be overthrown."

Albert Einstein*
1897-1955

2.1.1 Equilibrium constants

The thermodynamic parameter from which equilibrium concentrations are usually calculated is called the equilibrium constant. This fixed relationship between equilibrium concentrations was established experimentally by Guldberg and Waage between 1864 and 1879 as their "law of mass action" [1939]. However, it was only towards the end of this period that Horstmann and van't Hoff, independently, showed that it could be derived from the second law. [639]. A theoretical basis is important because it justifies the use of equilibrium constants to extrapolate into regions of such low concentration that data cannot be checked directly against experiment. The equilibrium constant expression arises from the fact that in closed systems under given circumstances of temperature and pressure, the Gibbs free energy must be a minimum. This may be expressed alternatively as $dG_{T,P} = 0$.

* "Autobiographical Notes" in Albert Einstein: Philosopher - Scientist, ed. E.A. Schilpp. [Harper & Row, New York] 1959.

Equation 2.1 is obtained when this condition is applied to the following generalized reaction.

$$aA + bB + \ldots\ldots \quad = \quad lL + mM + \ldots\ldots$$

$$\frac{\{L\}^{l} \cdot \{M\}^{m} \ldots}{\{A\}^{a} \cdot \{B\}^{b} \ldots} \quad = \quad K \qquad (2.1)$$

K is the equilibrium constant and the braces denote the thermodynamic activity of each species with respect to a given standard state. Although these terms are fundamental to the study of metal-ligand equilibria in aqueous solution, the literature of this field shows they are often misunderstood. Accordingly, certain aspects of each which are assumed throughout this thesis are outlined below.

The standard state for any solute species is that reference solution against which changes in the Gibbs Free Energy and other thermodynamic quantities can be measured. The activity of the solute species is defined in terms of the change in chemical potential (or partial molar free energy), μ, according to equation 2.2 [639].

$$\mu_i \quad = \quad \mu_i^{\circ} \quad + \quad RT \ln a_i \qquad (2.2)$$

Here, a_i is the activity of the solute species, μ_i° its chemical potential in the standard state, R is the universal gas constant and T is the absolute temperature. The chemical potential, μ, is defined in terms of the change in Gibbs Free Energy, G, with respect to the number of moles, n_i, under conditions of constant temperature, T, and pressure, P, by equation 2.3.

$$\mu_i = \left(\frac{\delta G}{\delta n_i}\right)_{T,P,n_1,n_2} \qquad (2.3)$$

The concept of activity was introduced by Lewis to represent the departure from ideality which is exhibited by real solutions [1939]. This deviation may be quantified as an activity coefficient, f, which relates the activity of a species to its concentration, c, as shown in equation 2.4.

$$f_i = \frac{a_i}{c_i} \qquad (2.4)$$

Conversely, the activity of an ideal solution is given by its concentration. In thermodynamic studies it is often preferable to express concentrations in terms of molality since this unit is independent of temperature and pressure. Molarities, on the other hand, give properties which are most easily interpreted in terms of molecular theories and which permit one to ignore the solvation of solute species [1941]. So there are also good grounds for choosing molarity as the concentration scale and this has been adopted throughout the present work.

The standard state is thus defined as that usually hypothetical solution at unit molar concentration whose properties are assumed to be ideal [639,1941,1942]. The reason for this rather abstract specification is simple: it makes the chemical potential such that the activity given by equation 2.2, becomes equal to the concentration (in the

appropriate units) as infinite dilution is approached. The nature of the standard state has no thermodynamic significance so a choice can be made on the basis of convenience. The effect of different choices merely alters the value of the equilibrium constant without affecting its constancy [639].

Another way of looking at this is to consider what happens when the concentration of a solute species is decreased towards infinite dilution [1942]. Its behaviour becomes progressively more ideal because there is a diminishing interaction between individual solute species. Hence the environment experienced by the solute becomes constant and the activity coefficient of the species approaches a limiting value which is unity if there are no components in the solution other than the solute and solvent in question.

It follows that the equilibrium constant shown in equation 2.1 can be obtained hypothetically by measuring the reactant and product concentrations in equilibrium as infinite dilution is approached. The question which must now be addressed is how this can be achieved in practice.

On the basis that interactions between solute species in solution give rise to non-ideal behaviour, it is easy to appreciate that electrical attractions and repulsions between ions must have a major effect on the properties of electrolyte solutions. Indeed, it was shown experimentally by Lewis and Randall that the activity coefficients of ions depend primarily on their charge and on the total concentration of all the charged species present in the solution. Other

contributions to the ion's chemical nature have very much less effect [1941]. They, therefore, introduced the concept of ionic strength, I, which is defined by equation 2.5.

$$I = \frac{\Sigma c_i Z_i^2}{2} \qquad (2.5)$$

where c_i is the concentration of the ith ion of charge Z_i. It follows that the activity coefficient of a solute species is approximately the same in all dilute solutions having a given ionic strength [1939].

In 1923 the Debye-Hückel limiting law was published [1943]. This showed how the activity coefficient of an ion in solution was expected to vary according to the ionic strength of the medium. The expression, given as 2.6, treats each ion as a point charge surrounded by a surplus of ions with opposite charge. At 25°C,

$$\log f_i = -0.5091 \, Z_i^2 \sqrt{I} \qquad (2.6)$$

Clearly, this simplified approach can only rationalise the gross effect of coulombic interactions in solution; the adjective "limiting" indicates that predicted and observed behaviour converge as the concentration of the solute is diminished toward infinite dilution. In practice there is good agreement up to ionic strengths of about 20 mmol dm^{-3} [70]. Various extensions to the limiting law which primarily aim to take account of the physical size of each ion have subsequently been developed. These can accurately predict experimentally determined activity coefficient values in

solutions with ionic strengths as high as 100 mmol dm^{-3} [1939]. Attempts to apply interionic attraction theories to solutions of mixed electrolytes, however, have met with limited success [71].

The Debye-Hückel theory substantiates the ideas of Lewis and Randall which led them to introduce the concept of ionic strength. It also provides a convenient way of extrapolating measured activities back to infinite dilution when it is not feasible to do so experimentally. However, for many systems (especially those of biological significance) it is not possible to work at the exceedingly low concentrations that this approach properly requires [1941]. As discussed in Section 2.1.3, moreover, the relevance of this procedure is open to question. An alternative is to employ a solution whose ionic strength is maintained constant by the addition of a "background" electrolyte. Such an approach assumes that the activity of a solute species under these circumstances will not vary as long as changes in its concentrations are very much smaller than those of the total electrolyte in solution. It is thus possible to determine equilibrium constants for reactions taking place in the electrolyte medium. The only requirement is that the actual activities of each species participating in the reaction can be ascertained. For practical purposes, however, it may be simpler to measure "stoichiometric" constants defined in terms of concentration, as shown in equation 2.7.

For the general reaction

$$aA + bB + \ldots\ldots = lL + mM + \ldots\ldots$$

$$K^* = \frac{[L]^l.[M]^m\ldots\ldots}{[A]^a.[B]^b\ldots\ldots} \qquad (2.7)$$

where K^* is related by expression 2.8 to the thermodynamic equilibrium constant K (defined in equation 2.1) as long as the activity coefficients, f, of each species remain constant.

$$K^* = \frac{K.f_A.f_B\ldots\ldots}{f_L.f_M\ldots\ldots} \qquad (2.8)$$

There are now two possibilities to consider [1942]. Firstly, if the standard state is defined in terms of pure water as the solvent, the activity coefficients are constant but not equal to unity. Their value mainly reflects the high ionic strength of the solution with respect to the standard state. Note that reference is still made to infinite dilution of the solute species (not the background electrolyte!) but one must consider this infinite dilution in the ionic medium with respect to the same condition in pure water. Measured parameters must therefore be corrected using a Debye-Hückel-type function to obtain "thermodynamic values". Alternatively, one can define the standard state in terms of the electrolyte solution as the solvent. Then the activity coefficient for the solute species at infinite dilution is put at unity.

Initial increases in the concentration of solute will have no significant effect on ionic strength because of the swamping excess of background electrolyte so no Debye-Hückel corrections are applicable. Furthermore, as long as the activity coefficients remain constant, "stoichiometric" quantities such as K^* in equation 2.7 and 2.8 are equivalent to the corresponding "thermodynamic" values.

It might seem that the choice of electrolyte solution as the solvent in the definition of the standard state is so convenient that it would be adopted universally. That this is not the case is partly due to a lack of agreement concerning the salt which should be used. A large diversity of background concentrations have also been employed. More fundamentally, one cannot justifiably assume that a change in ionic strength is the only reason for non-ideal behaviour [1941]. Thus, the activity coefficients of solute species in dilute but experimentally realistic concentrations may deviate from their values at infinite dilution but still appear constant over a fairly wide range of concentrations. Strictly, the assumptions should always be tested by extrapolation to infinite dilution before results are claimed to have thermodynamic significance. When this is not possible, it must be shown that the equilibrium quotient remains constant over as large a variation in reactant and product concentrations as is practicable [1941]. To illustrate the difficulties involved, it has been suggested that the structure of a concentrated electrolyte solution such as sodium perchlorate at 3 mol dm^{-3} may be seriously deranged

at very low solute concentrations because almost all the water present in such solutions participates in the solvation of the electrolyte ions; this implies a dramatic change in the environment of solute species at these very low concentrations so that their activity coefficients may rapidly or suddenly deviate from unity and therefore thwart attempts to extrapolate back to infinite dilution.

One further matter which must be dealt with in this section concerns the interaction between solute species and the ions of an electrolyte solvent. As any such interaction obviously affects the environment of the solute it must contribute to its activity. Much attention has been focussed on background salts that are as "inert" as possible. Potassium nitrate and sodium perchlorate have emerged as two of the most commonly used. Of course, the "non-coordination" of anions such as nitrate and perchlorate is a relative phenomenon in which their poor ability to donate electrons to many metals is contrasted with respect to water molecules [392]. Thus, measurements in solutions of "inert" salts give the closest approximation to a solution in the pure solvent because only the effect of ionic strength need then be taken into account. In practice, the nature of the electrolyte always has some influence [377], but the less the interaction the more applicable Debye-Hückel functions become. However, it is clear that this criterion is not relevant when the standard state is defined in terms of the electrolyte solution as the solvent. Under these circumstances, interactions with the ions of the electrolyte are simply part of the interaction of the solute with the solvent.

So, once again, a suitable choice of standard state and in particular of the background salt to maintain ionic strength, removes many thermodynamic difficulties. The only effects which need cause any concern are those leading to non-ideal behaviour as mentioned in the previous paragraph.

2.1.2 Competitive equilibria in aqueous solution

It is difficult to find examples set in a variety of chemical contexts which are simple but illustrate the concept of equilibrium adequately. It has accordingly become popular in modern textbooks to treat labile metal complexing reactions amongst the problems and exercises dealing with the law of mass action. However, this presentation is prone to be over-simplified, particularly in the neglect of the many competitive reactions which are usually involved. Such omission often leads to ill-defined or overtly incorrect results. Indeed, only over the last decade has the application of high speed computers permitted even research workers to routinely take into account the many possible kinds of complex species [364,1807].

The powerful tendency of water to donate a lone pair of electrons to Lewis acid acceptors means that metal ions dissolved in water will generally exist with their coordination sites fully occupied by solvent molecules. Other complexing agents added to the solution must then compete with water in order to bond to the metal. The extent to which their coordination can take place depends upon the equilibrium which is set up between the aquated metal ion species and the complex

formed by the added ligand. This is shown in the following example where charges have been omitted for the sake of simplicity and generality.

$$M(H_2O)_6 + L \rightleftharpoons ML(H_2O)_5 + H_2O$$

In general, successive replacement of water molecules can occur so that a whole series of competitive equilibria arise. As the solvent implicitly occupies all sites which are not coordinated to other ligands, an abbreviated representation of the step-wise reactions is often used.

$$M + L \rightleftharpoons ML$$
$$ML + L \rightleftharpoons ML_2$$
$$ML_2 + L \rightleftharpoons ML_3$$

Each of the above reactions is characterized by an equilibrium constant that quantitatively represents the avidity with which the ligand binds to the metal compared with the solvent under specified conditions. Assuming that the ionic strength of the solution is invariant, stoichiometric constants for each reaction can be formulated as follows.

$$K_1^* = \frac{[ML]}{[M][L]} \quad ; \qquad K_2^* = \frac{[ML_2]}{[ML][L]} \quad \text{etc.} \qquad (2.9)$$

By convention these are usually expressed as "step-wise formation constants", $\beta^{M,L}$, by writing each as the cumulative product of the individual reaction constants, K^*. Thus

$$\beta_1^{M,L} = K_1^* = \frac{[ML]}{[M][L]}$$

$$\beta_2^{M,L} = K_1^* K_2^* = \frac{[ML]}{[M][L]^2} \qquad (2.10)$$

In this way the concentration of a complex species is defined in terms of its formation constant and the product of its components' concentrations, each raised to the power of the appropriate stoichiometric coefficient.

It is important to emphasise that a competitive equilibrium has been described in which every species exists at the expense of the others. The outcome depends on a variety of factors but particularly on both the absolute and relative concentrations of the components. Another critical element which should never be forgotten (although it often is) is the solution's free hydrogen ion concentration. Electron donor sites on ligands can bind protons as well as metal ions so there is yet another competition in solution which determines the concentration of free complexing species available. This series of equilibrium reactions may be represented by

$$L + H \rightleftharpoons HL \quad ; \quad \beta_1^{H,L} = \frac{[HL]}{[H][L]}$$

$$HL + H \rightleftharpoons H_2L \quad ; \quad \beta_2^{H,L} = \frac{[H_2L]}{[H]^2[L]} \qquad (2.11)$$

In addition to this, hydroxyl ions are much more powerful electron donors than water molecules so alkaline conditions favour metal ion hydrolysis at the expense of all the other metal complexes present.

In general, then, a plethora of species must be expected whenever solutions of a metal ion and of a ligand are mixed together. The situation is even more complicated by the possibility of polynuclear complexes such as M_2L_2 and of so-called "mixed" species where one or more protons or hydroxyl ions are appended to or lost from the usual step-wise metal complexes. For example, these may be represented as MLH, MLH_2, MLH_{-1} and MLOH. To accommodate all of these species a general formulation of the formation constants applicable to one metal and one ligand systems has been adopted. For the general reaction,

$$pL + qM + rH \rightleftharpoons L_p M_q H_r$$

$$\beta_{pqr}^{L,M,H} = \frac{[L_p M_q H_r]}{[L]^p [M]^q [H]^r} \qquad (2.12)$$

It is pertinent to note at this stage that in all of the mathematical expressions dealing with equilibria in aqueous solution, the hydroxyl ion is equivalent to a negative hydrogen ion contribution. Thus, MLH_{-1} and MLOH represent the same complex stoichiometry. This arises out of the relationship between the dissociation of water and the other reactions in the solution by virtue of water's role in the definition of the standard state. Those reactions in which water is involved are transparent to the others. Hence, formation constants may be expressed in terms of hydroxyl ion concentration or instead

may be related by the dissociation constant to the hydrogen ion concentration.

$$[OH] = K_w[H]^{-1} \qquad (2.13)$$

The basic mathematical problem to be solved in the simulation of metal-ligand equilibria is how to calculate the free concentrations of each component given the total concentrations and all the necessary formation constant values. Once the free concentrations are known, all the complex species concentrations can be readily evaluated from the formation constants. This matter will be dealt with in detail in Section 2.2 but certain elementary aspects are best introduced at this stage. For each component, a "mass balance" equation can be written in which the concentration of the component is summed over all the species.

$$\begin{aligned} T_L &= [L] + [HL] + [H_2L] + [ML] + 2[ML_2] + \ldots\ldots \\ T_M &= [M] + [MOH] + [ML] + [ML_2] + 2[M_2L_2] + \ldots\ldots \\ T_H &= [H] - [OH] + [HL] + 2[H_2L] + \ldots\ldots\ldots\ldots \end{aligned} \qquad (2.14)$$

It may be noted, incidentally, that the hydrogen ion mass balance constraint is often represented by the equivalent but unsystematic electroneutrality condition [106]. By introducing the equilibrium constants, these expressions resolve into a sum of terms containing only the free concentrations as unknowns.

$$T_L = [L] + \beta_{101}[H][L] + \beta_{102}[H]^2[L] + \beta_{110}[M][L] + 2\beta_{210}[M][L]^2 + \ldots$$

$$T_M = [M] + \beta_{01-1}[M][H]^{-1} + \beta_{110}[M][L] + \ldots\ldots$$

$$T_H = [H] - \beta_{00-1}[H]^{-1} + \beta_{101}[H][L] + \ldots\ldots\ldots \qquad (2.15)$$

It is thus possible to solve for [M], [L] and [H] simultaneously and hence obtain the equilibrium distribution. If any of the free concentrations are known instead of a total value (as is often the case with hydrogen ions) then the number of equations to be solved is simply reduced. Conversely, when a free concentration is measured in a system where all the total concentrations are already known, the problem becomes over-defined and the additional information can be used to estimate the values of the formation constants. This is of considerable relevance to the discussion in Chapter 3.

2.1.3 Procuring formation constants for bioinorganic systems

In practice, one takes a slightly different perspective to that presented in Section 2.1.1 when applying thermodynamic theory to bioinorganic formation constants. The reason is that biological fluids usually exist at relatively high ionic strengths with sodium chloride as the dominant electrolyte. Attempts to calculate equilibrium concentrations in such media are obliged to take account of this whether or not it is convenient or indeed even permissible from a thermodynamic viewpoint! Thus, there is less stress on the stringent

requirements of a "thermodynamic" approach. After all, worthwhile answers to real questions seldom lie in semantics! The question is not so much about which standard state should be adopted but rather to what extent various standard states and other thermodynamic functions are valid in the biological situation. Even when it transpires that they are not strictly applicable, they may nevertheless be good enough to yield sensible results. Moreover, by comparing and contrasting the actual situation with the theoretical one it is often possible to expose the most relevant parts and thus find the most vulnerable flaws in a given method.

The vast majority of formation constant measurements reported in the literature have assumed, for purposes of defining their standard state, that water is the solvent. This reflects the thermodynamic motivation behind the research, especially the earlier work, which was not specifically aimed at determining equilibrium concentrations in applied situations. Unfortunately, there are few practical areas where thermodynamic equilibrium constants of metal-ligand reactions at infinite dilution in water are relevant. So there is now a growing trend towards experiments designed with some ultimate objective in mind.

The most apposite temperature for formation constant measurements in a bioinorganic context is probably that of the human body, namely 37C. There is no theoretical objection with such a choice but a few experimental complications may be thereby introduced. For the most part, however, the accuracy of results will not be adversely affected by this because other factors tend to be much more critical.

The need to use sodium chloride solution at 150 mmol dm^{-3} as the most pertinent reference for biological fluids (if not as the solvent in the definition of the standard state) is much more problematical. Ironically, experimental procedures are greatly simplified because this salt can be obtained in a pure and dry condition, relatively easily. Thus, metal-ligand systems which do not interact unduly with sodium chloride are best studied directly in this medium. An argument can be made for doing likewise with all metals and all ligands of biological interest but the majority of authorities probably feel that an indirect approach is to be preferred [286]. When either the ligand binds sodium ions or the metal binds chloride to a significant extent, the metal-ligand equilibria can first be studied in a more inert background medium. The effect of sodium chloride can then be quantified in the same background electrolyte and this information used to calculate the various interactions in the biological fluid. The advantage of this approach is that the primary metal-ligand formation constants can be readily compared with values in literature for the same and other metal-ligand systems.

In this work the database of formation constant values assembled for the computer simulation of the equilibria in blood plasma has been substantially extended by incorporating values for about 30 exogenous chelating agents. Data has been critically selected from the literature and treated in the same way as previously described [736]. The account below outlines this procedure again but only very briefly and the reader is referred to the original work for more detail. Two further metal ions,

Ni(II) and Fe(II), have been added to the earlier list of Ca(II), Cu(II), Fe(III), Pb(II), Mg(II), Mn(II) and Zn(II). So values for Ni(II) and Fe(II) with the 40 naturally occurring ligands that are considered have also been provided. In addition, the earlier database has been rigorously updated and accordingly a number of extra formation constants have been included. The whole list of 1700 binary complex species appears as Appendix A2. This is to be compared with the set of under 1000 which was prepared for the original simulations [736].

Whenever sufficient data has been available, the integrated form of the van't Hoff equation has been used to correct formation constants to 37 C standard comparable temperature. This is shown in equation 2.16 [639].

$$\ln \left(\frac{K_2}{K_1}\right) = \frac{\Delta H^\circ}{R} \left(\frac{T_2 - T_1}{T_1 T_2}\right) \qquad (2.16)$$

Here, ΔH°, the standard change in heat content, is assumed to be independent of temperature, R is the gas constant and T_1 and T_2 are the different temperatures in Kelvin. (K_2/K_1) is the relative change in the equilibrium constant. When equation 2.9 could not be used empirical corrections based on various experimentally determined trends were employed instead [504].

Empirical methods have also been adopted to correct for changes in ionic strength. With the data available, Debye-Hückel type corrections are almost invariably inapplicable. Fortunately, Gergely *et al* have studied the effect of both ionic strength and the nature of the electrolyte on the equilibrium constants of the complexing reactions between some amino acids and transition

metal ions [377]. This work has provided a valuable benchmark from which the direction and magnitude of adjustments can be judged fairly precisely. Even more relevant in the present context has been the fact that the majority of medical chelating agents have been thoroughly investigated under a wide variety of experimental conditions.

Perhaps the most controversial aspect about the formation constant database that has been assembled is the procedure adopted when the literature is found to be in error, ambiguous, contradictory or incomplete. These circumstances often arise because of inherent experimental limitations which prevent the categorical definition of complexing species which exist in solution. This subject is further discussed in Section 3.3. In selecting formation constants, an attempt has been made to adhere to the following policy.

(i) Averages are taken when there is little to distinguish between two or more values obtained by different research workers.

(ii) Formation constants published by the most active, well established laboratories are used in preference to others. However, when sets of formation constants obtained in this way are incomplete (in the sense that they do not include all species which might reasonably be expected to exist in solution), then the set is supplemented by values from additional sources.

(iii) Educated guesses are made to obtain formation constants which are not otherwise available from the literature but which seem certain to exist under some circumstances in aqueous solution.

Such estimates are derived from various observed chemical trends, most notably relying on the relationship between ligand basicity and complex stability [364,505] and the Irving-Williams series for comparing the complexing ability of the first row, divalent transition metal ions [619].

The motive behind this approach has primarily been to ensure that complex species which may be important *in vivo* are not omitted because they are experimentally difficult to detect. A degree of incompatibility between the formation constants used is thus introduced but the associated errors are likely to be small because species at or below the limit of detectability *in vitro* will only seldom become significant in biological fluids. On the other hand, very serious errors indeed may occur if a complex is neglected. It should be remembered that to omit a complex species means that in all simulations it is treated as though its formation constant is zero; it is usually possible to estimate a better value than that.

There is one further element which combined with the above rules makes the procedure fail-safe. It is that the results of all simulations are evaluated in terms of the reliability of the most pertinent input data. Thus, each complex species is subjected to further scrutiny whenever it occurs at significant concentrations in a computer model or when it may do so if its formation constant is reasonably increased. This highlights those values which are in greatest need of revision and ultimately determines whether they warrant experimental investigation. A good example of how this works is described in Section 4.2.1.

Although the vast majority of binary formation constants in the database have actually been measured under one circumstance or another, this is not the case for the ternary complexes. Special methods have therefore been devised for including this kind of species in the computer models. Basically, most values are calculated on the assumption that if parent binary complexes, MA_2 and MB_2, exist then the ternary species MAB will also occur when both ligands are mixed in solution with the metal ion. A statistical argument [88] predicts that the formation constant of such species can be estimated by equation 2.17.

$$\log \beta^{MAB} = \frac{[MAB]}{[M][A][B]}$$

$$= \tfrac{1}{2}(\log \beta_2^{M,A} + \log \beta_2^{M,B}) + \log 2 \qquad (2.17)$$

Clearly, this theoretical calculation of ternary formation constants must be subordinate to adjustments based on experimental information and these, in turn, are only appropriate when the value in question has not been measured under biological conditions of temperature and ionic strength. The computer program MIX was written primarily with a view to assembling ternary formation constants in accordance with these hierachical requirements. However, for convenience sake, the program also selects those binary formation constants in the database needed by a model. The main alterations to the original structure of the program have been made so as to permit the user to override these automatic procedures. Thus, one is now able to prevent

constants being selected on the basis that they include one or more specified components. Other values can be substituted instead, if desired. This is particularly useful to those who have determined a set of constants experimentally and wish to establish the extent to which their new values affect previous models. No alterations to the database need be made. Another major innovation which has been made to program MIX concerns the elimination of some ternary complex species. Many of the calculated formation constants produced by the program are such that the complex is certain to occur at insignificant levels in the simulated solution. These can be identified and so omitted by program MIX on the basis of a preliminary calculation using approximate values for each free component concentration. In this way, about 9000 complex species are routinely considered for the blood plasma model described in Chapter 4 but the number is reduced to 5000 when the actual simulation is performed. A detailed description of program MIX, its input instructions and FORTRAN listing can be found in Appendix A3.

2.2 The simulation of multicomponent systems

An extraordinarily large number of mathematical methods have been developed to calculate equilibrium concentrations. It is possible to reference only some of the papers which are relevant to one or another computer application [8-14, 17, 84, 85, 89, 91, 92, 110-112, 119, 128, 204-206, 215, 216, 219, 245, 258, 259, 327, 510, 567, 825, 915, 1553, 1622, 1775, 1886]. Many of these approaches, however, deal with only specific equilibrium systems and thus fail to make the most of computer facilities that are nowadays commonly available. The relative costs of human and computer time have become such that modern simulation programs must treat the whole gamut of systems from simple ligand protonation to the most complicated, polynuclear complex formation.

There are two well-established and commonly used computer programs for simulating general metal-ligand equilibria in solution. The first was called COMICS (for Concentrations Of Metal Ions and Complexing Species) [111] and the second is known as HALTAFALL (from the Swedish "halta" meaning 'concentration' and "falla" meaning 'precipitate') [112]. The essential differences between these programs can be summarised by saying that although HALTAFALL tends to be less efficient than COMICS for those systems which can be treated by them both, the former can be applied to a much wider range of problems. As its name implies, HALTAFALL can deal with systems having more than one phase. On certain occasions this can be a very useful feature but it is not really relevant to the present work. Of fundamental import, however, is the fact that HALTAFALL can calculate free hydrogen

ion concentrations from a given total concentration. COMICS cannot. Hence, HALTAFALL must be used to simulate titrations. COMICS deals only with aqueous solution and requires the free hydrogen ion concentration as input. When this is known, as in most equilibrium simulations of biofluids, the approach adopted by COMICS is simpler both to use and to understand so it is this which will be dealt with in detail.

Another program which should be mentioned at this stage is ECCLES (for Evaluation of Constituent Concentrations in Large Equilibrium Systems) [736,1250]. Like COMICS, it is restricted to situations where the free hydrogen ion concentration of the solution is known. However, it has a variety of features which recommend its use even in the case of single metal and single ligand simulations. It can accept the free concentration of any component whereas COMICS cannot. It can also scan either the free or the total concentration of any component. COMICS can only treat a series of different pH values. A full description of program ECCLES, the instructions for preparing input and a FORTRAN listing appear in Appendix A3. The remainder of this Chapter is devoted to a brief outline of how ECCLES works (with emphasis on the improvements which have been made to the original version) and what advantages it offers in the simulation of large multicomponent systems compared with COMICS.

2.2.1 Generalized simulation techniques

The nomenclature, symbols and conventions used in Section 2.1.2 for writing the formation constant and mass balance expressions of binary equilibrium systems develops into a very cumbersome structure once four or more components are considered. It is therefore desirable to set out a more general mathematical representation by introducing a new notation and writing the two equations shown below: 2.19 represents the formation constant expression introduced as 2.12 and 2.20 is equivalent to the mass balance relationships depicted as 2.14.

$$S_j = \beta_j \prod_i X^{k(i,j)} \qquad (2.19)$$

$$T_i = X_i + \Sigma_j S_j.k(i,j) \qquad (2.20)$$

The meaning of these generalized symbols is as follows.

S = complex species concentration
X = free component concentration
T = total component concentration
β = cumulative (overall) formation constant
i = component index
j = species index
k = stoichiometric coefficient matrix

The value of k is the number of times the ith component appears in the jth species. Thus, the large majority of the array's elements will be zero. This important point is highlighted by the fact that even in a system of one metal ion and two ligands, the binary complex species are commonly five or ten times as numerous as ternary ones. The discrepancy is worsened as the number of components in the system increases.

The chief reason for rewriting program ECCLES was to reduce its core storage requirement. The original version was itself very economic in this respect: a considerably smaller three dimensional array had been used instead of the two dimensional k matrix. A set of number pairs was thus stored for every complex species to specify both the stoichiometric coefficients and their corresponding component indices. The latter information is implied by the position of an element in the k matrix. In the three dimensional array only 5 x 2 elements were devoted to each complex species so for every component in the system over ten in number, one element was saved per complex species. For example, in the blood plasma model having 5000 complexes from about 50 components, this meant an economy of just less than 200K computer words. This is larger than the total memory capacity of many computers. However, the efficiency did not only encompass core storage. Large scale savings of computer time were also made because this enormous number of irrelevant entries no longer had to be processed.

However, this approach did not go as far as it might have done. Up to 40 per cent of the three dimensional array still remained unutilized. When it became necessary to further reduce the size of the program, this wastage was an obvious target. It is almost entirely eliminated in the new version of ECCLES by employing a one dimensional array rather than the three dimensional one. In this, the component indices are stored alone. When a component appears more than once in a complex species this is accommodated by storing the component index an appropriate number of times. Thus, except for the room at the end (which depends solely on the total size specified), the one dimensional

array is fully utilized. Once again, this means a saving of both computer time and of storage requirements. This is in spite of some overheads which the additional "bookkeeping" introduces.

One serious disadvantage of the new method of storing the information about stoichiometric coefficients is that it becomes impossible to treat negative coefficients for the hydrogen ion in the conventional manner. Clearly, one could indicate the reciprocal of a free component concentration, for example, by a negative value for the component index. However, this would introduce an unwarranted amount of extra processing at a time-critical stage in the iterative calculations. It was, therefore, decided that, on input, ECCLES should convert all complexes with negative hydrogen ion subscripts into the corresponding hydroxy species and make a suitable adjustment to the formation constant (see Section 2.1.2). This is easily done amongst the multitude of input checking procedures which the program implements. A slight inconvenience to the user is that these species are not converted back into their original form on output, mainly because the program is unaware of which hydroxy species should be so treated. Of course, this artefact also means that hydroxide ion must be included in the input of free concentrations.

2.2.2 Iterative procedures for large equilibrium systems

Once a set of formation constants have been specified, the mass balance equations for the equilibrium system become uniquely defined. In other words, a set of n non-linear equations is obtained (by substituting equation 2.19 into equation 2.20) where n is the number of components whose total concentration is known and whose free concentration is to be determined. It is not generally possible to solve these equations directly because of their non-linear nature. The answer lies in successively improving some initial estimates of each free component concentration. Such an iterative approach must be based on a comparison between the real, known total concentrations and the corresponding calculated values that are produced by equations 2.19 and 2.20 from the current, best estimates. When agreement is reached, i.e. the difference between the real and calculated quantities becomes less than a predetermined tolerance, the iteration is said to have converged.

The key to all numerical techniques of this kind lies in the method by which the estimates are varied towards the final solution. They clearly require well devised formulae that will not only avoid divergence but also utilize a minimum of computation. This is important because hundreds of iterations may be required to obtain one set of equilibrium concentrations. A titration involves tens or hundreds of such simulations and when it comes to the determination of formation constants described in Chapter 3, titrations often have to be processed hundreds if not thousands of times. So even in these days when the cost of

computer time is falling by an order of magnitude every five years, inefficiency at the level of the iterative formula can be expensive.

On the other hand, there tends to be a balance between the speed of these computational algorithms and their ability to converge under adverse conditions, i.e. their robustness. When an iterative procedure fails a great deal of calculation can be wasted. Moreover, the computer program may then simply be unable to cope with the problem at hand. So it is unprofitable to sacrifice robustness for rapid convergence.

Another aspect which must be mentioned is that many numerical techniques for non-linear equations have been devised which utilize extremely large amounts of computer memory. For example, by calculating and storing values for derivatives and second derivatives at every point in a titration it is sometimes possible to greatly enhance the rate of convergence. Computer technology has reached the stage where machines with very large internal core storage and/or virtual memories are becoming commonplace so this is an acceptable trend. However, there are limits even to modern facilities which prevent this kind of approach being used with the very large simulations of metal-ligand equilibria in biofluids that are described in Chapter 4.

It should also be said that most general methods for solving non-linear equations run into convergence difficulties as the number of equations increases [216]. It is thus necessary to look for specific mathematical methods for treating the general chemical problem. One that comes readily to mind is called successive approximation. This is a somewhat ambiguous name for

it could equally well apply to other iterative methods. In its simplest form it means that the equation $g(x) = 0$ can be solved for x using a formula constructed by rearranging the equation into a form where $x = f(x)$. Thus, one obtains equation 2.21.

$$x_n = f(x_{n-1}) \qquad (2.21)$$

A reasonable guess, x_0, is used to obtain an improved estimate, x_1, and this is then substituted back into 2.21 to get an even better value, x_2 [1945].

Not all rearrangements of $g(x) = 0$ will produce formulae $x = f(x)$ that converge. In fact, it is necessary that the derivative of $f(x)$ in the interval between the initial estimate and the true value is always less than unity. The smaller the derivative is, the more efficient the formula becomes [1945]. Successive approximation techniques are rarely selected for their speed, particularly if this is to be judged by the number of iterations required for convergence. However, they possess three main advantages: "robust" formulae can usually be constructed, they usually have relatively small store core requirements and they involve relatively little computation. For these reasons, they are most valuable for simulating very large systems.

The original version of ECCLES used three iteration formulae. The main one was identical to that used by program COMICS and is shown as equation 2.22.

$$^{n}X_m = {}^{o}X_m \left(\frac{^{r}T_m}{^{c}T_m} \right)^{\frac{1}{2}} \qquad (2.22)$$

Here, m is a specific value of i, the component index, and the superscripts n, o, r and c denote the 'new', 'old', 'real' and 'calculated' quantities respectively. Thus, the square root of the ratio of the known total concentration to its calculated counterpart is used to adjust the free component concentration.

For the initial iterations (when the estimates are generally very poor) a more complicated formula was derived. This is shown as equation 2.23.

$$ {}^{n}X_m = \frac{{}^{o}X_m \cdot {}^{r}T_m}{{}^{o}X_m + \Sigma_j [G'_j \cdot S_j \cdot k(m,j)]} \qquad (2.23) $$

where

$$ G'_j = \frac{\prod_i \left(\frac{{}^{r}T_i}{{}^{c}T_i} \right)^{k(i,j)/2}}{\left(\frac{{}^{r}T_m}{{}^{c}T_m} \right)^{\frac{1}{2}}} $$

The formula requires considerably more calculation than does 2.22 but it proved worthwhile in the opening stages.

Interestingly, certain properties of equation 2.22 become apparent from the behaviour of equation 2.23 as it approaches the solution. Under these circumstances, the value of G'_j tends to unity and thus the denominator in 2.23 approaches the total calculated concentration. This suggests that formula 2.24 will become increasingly efficient as it nears the solution and it was accordingly introduced into program ECCLES to be used alternatively with 2.22 after the first 25 iterations.

$$ {}^{n}X_m = {}^{o}X_m \left(\frac{{}^{r}T_m}{{}^{c}T_m} \right) \qquad (2.24) $$

However, it was also shown that 2.24 could normally be expected to "overshoot" the solution, i.e. to convert an estimate that was too large into one that was too small or *vice versa*. Iteration procedures in which equation 2.24 was introduced prematurely could thus oscillate back and forth with very poor or even no convergence capability. On the other hand, it was noted that by rewriting equation 2.22 as equation 2.25, the denominator is the geometric mean between ${}^{c}T_m$ (which would overshoot) and ${}^{r}T_m$ (which leaves X_m unaltered).

$$ {}^{n}X_m = {}^{o}X_m \cdot \frac{{}^{r}T_m}{\sqrt{{}^{r}T_m \cdot {}^{c}T_m}} \qquad (2.25) $$

It was thus proposed that the tendency of 2.24 to overshoot and to oscillate was curbed in equation 2.22.

The three-tier algorithm used in program ECCLES as described above has proved very successful in a wide variety of metal-ligand equilibrium simulations. Convergence was often found to be remarkably rapid, particularly in the case of the blood plasma models when, typically, the solution was reached after fewer than 30 iterations. However, during a project to determine the formation constants of the zinc-cysteinate system under biological conditions of temperature and ionic strength (see Section 4.2.1) a serious weakness was uncovered. Divergence was found to occur whenever polynuclear complexes of the type

M_2L_3 or M_3L_4 predominated in the solution. Neither formula 2.22 nor formula 2.24 could cope under these circumstances.

The reason for these failures emerged only after some investigation. Ginsburg [825] had suggested that equations like 2.22 and 2.24 could be expressed in the more general form of equation 2.26.

$$ {}^{n}X_m = {}^{o}X_m \left(\frac{{}^{r}T_m}{{}^{c}T_m} \right)^{1/p_m} \qquad (2.26) $$

He argued that the optimum value of p_i in this expression was related in a complicated way to the stoichiometry of all the complexes in the equilibrium system. That this is likely to be so can easily be seen from the simple case where only one species predominates and only one free component concentration is unknown. Then one can write equation 2.27.

$$ T_m = S_j\, k(m,j) = k(m,j)\beta_j \prod_i X_i^{k(i,j)} \qquad (2.27) $$

By cancelling all the known terms, one can obtain equation 2.28.

$$ \frac{{}^{r}T_m}{{}^{c}T_m} = \left(\frac{{}^{r}X_m}{{}^{o}X_m} \right)^{k(m,j)} \qquad (2.28) $$

Whence, an exact solution can be obtained as equation 2.29. Note the relationship between p_m in 2.26 and $k(m,j)$ in 2.29.

$$ {}^{r}X_m = {}^{o}X_m \left(\frac{{}^{r}T_m}{{}^{c}T_m} \right)^{1/k(m,j)} \qquad (2.29) $$

Ginsburg's mathematical analysis proved intractable and he was unable to derive the best value to use for p_m [825].

He arbitrarily suggested that $p_m = 5$ would be satisfactory. When this proposal was tested with a view to incorporating it into the new version of ECCLES, however, it was found to yield an inordinately slow rate of convergence. This is to be expected from discussion surrounding equation 2.24. By taking the fifth root of the $({}^{r}T_m/{}^{c}T_m)$ fraction, the factor by which the free concentration, ${}^{o}X_m$, is adjusted is bound to be closer to unity than when the square root of the ratio is used. It is thus apparent that the larger the value of p_m, the less likely the iteration is to diverge or oscillate but the more inefficient it becomes.

Consideration of equation 2.29 and the corresponding relationships for more complicated situations suggests that the optimum value for p_m is close to the maximum value of k(m,j) over those complex species which are the major contributors to the total calculated concentration, ${}^{c}T_m$. On these grounds, a revised iteration algorithm has been implemented in the new version of ECCLES. In particular, a fourth iteration formula (2.26) is gradually introduced if there is no convergence after 30 iterations. In this event, the values p_i are set to the highest value of k(i,j) found for those species which account for more than 1% of the current calculated total concentration. This is necessary because it was found that putting $p_i = \max(k(i,j))$ over all the species led to slow convergence.

The earlier iteration formulae are retained because of their proven efficiency with most systems. The only significant change has been to revise the calculation of G_j' in equation 2.23 to take account of the more robust formula 2.26. This is shown as equation 2.31.

$$G_j' = \frac{\prod_i \left(\frac{r_{T_i}}{c_{T_i}}\right)^{k(i,j)/p_i}}{\left(\frac{r_{T_m}}{c_{T_m}}\right)^{1/p_m}} \qquad (2.31)$$

Evaluated in this way, equation 2.23 acts as a slight convergence accelerator in the later stages of the iterative procedure. Accordingly, it is implemented every fifty cycles to perturb the approach to the solution.

In a large majority of cases, these changes have caused a small decrease in the rate at which program ECCLES converges. This is more than compensated by the additional robustness of the new four-tier algorithm.

CHAPTER THREE

COMPUTER SIMULATION IN THE DETERMINATION OF FORMATION CONSTANTS

3.1 The role of simulation in optimisation procedures

Optimisation is the process of determining those conditions which yield a best possible result. In the context of metal-ligand equilibria, this means finding the values of one or more constant coefficients in the mathematical relationships which define the system such that an optimum agreement between calculated quantities and their observed counterparts is achieved.

Those coefficients which are invariant with respect to a given set of data but which may alter in value from one experiment or one system to another are called parameters. They may be either "local" or "global" depending on whether they apply to part of the data set or to the whole of it. For example, in potentiometric studies, formation constants are said to be global parameters, whereas the initial concentrations and volumes of each titration are local ones. Parameters are to be contrasted with quantities that are systematically varied through the titration such as a free component concentration.

To recognise an optimum agreement, one requires a precise criterion to distinguish between different results. Formulated mathematically, such a criterion is called an 'objective function' [207]. Depending on the circumstances, its value may have to be either maximised or minimised. Often, the objective function will be based on the difference between observed and calculated quantities for, clearly, the smaller this is, the better the agreement. Each difference is known as a residual. These need

to be minimised over the entire range of data. By calculating a sum of squared residuals one obtains an overall criterion that neglects the sign of individual contributions. This is by far the most popular kind of objective function and it is embodied in all of the computer programs for determining formation constants that are discussed in this thesis. Such optimisation methods are collectively known as methods of 'least squares'.

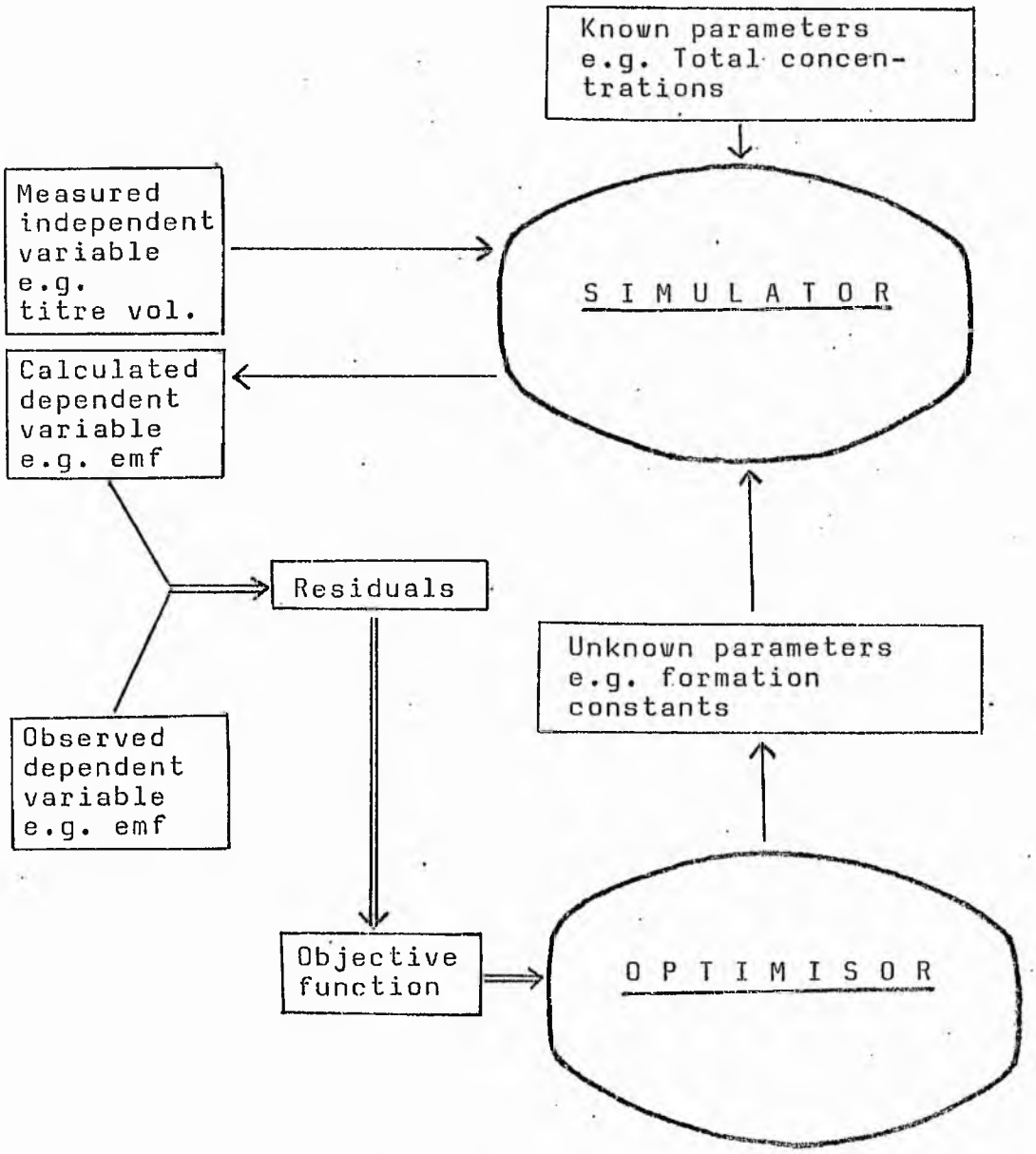
So widespread is the use of 'least squares' that every chemist is aware of at least one optimisation technique. This is associated with the statistical method called linear regression analysis by which the value of one quantity can be estimated from another by finding the best straight line relationship between them. By making certain assumptions about the quantities themselves and the errors involved in their measurement it is possible to determine not only the best values for the slope and intercept parameters but also their precision and the extent to which confidence in a linear relationship is experimentally justified. The 'least squares' optimisation can be done approximately by eye (using some graphical representation of the data) or by calculation. In the latter case, formulae are available which yield values for the best slope and intercept directly [1948]. Accordingly, very many chemical experiments have been designed specifically to produce data which is amenable to linear analysis. More complicated relationships are tackled by hand occasionally but unless the problem can be reduced to a linear form it is all too often regarded as intractable. With the advent of modern high speed computers, such an attitude is no longer justifiable.

A host of general numerical methods for locating the optimum in situations where one or more parameters are unknown have been developed [15,16,18,65,207,209,210,227,388,397,615, 1006,1769,1946]. A detailed discussion of their mathematical foundations or of their respective merits lies outside the scope of the present work. Suffice it to say that many employ iterative procedures which may also be applied to the solution of non-linear equations as discussed in Section 2.2.2. This overlap sometimes causes confusion between the calculations for optimisation and those for simulation. It may help to clarify the position if one remembers that a non-linear equation $g(x) = 0$ can be solved by varying the value of x in such a way that the absolute value of the function is minimised. On the other hand, simulations are commonly employed during optimisation procedures, especially when a simple mathematical expression for the relationship between two measurable quantities cannot easily be found. Instead, the relationship may be simulated by sequential application of a series of formulae until the values for all the variables in the system have been determined. Residuals can then be obtained by comparing the observed data with its corresponding calculated value. Hence, an objective function can be evaluated.

The role of simulation in optimisation procedures is depicted diagrammatically in Figure 3.1. This shows how a measured, independent variable can be used in a simulation to calculate an associated value for another variable quantity that

Figure 3.1

The role of simulation in optimisation procedures.



can also be directly observed. To do this, the whole set of parameters is required so if, in fact, a parameter is being determined a current estimate of its value must be employed. From the behaviour of the objective function when these estimates are changed, the direction of the optimum is ascertained by the numerical optimisation technique. The estimates are then varied accordingly. In other words, the optimising segment follows a trend which, on the basis of its previous experience, seems likely to further decrease the objective function value. The new parameter estimates are used to recalculate the dependent variable data and so the cycle is repeated. When every small variation in the parameter values causes the objective function to increase no more changes can be made and the optimisation is said to have converged on the best possible result.

In principle, the algorithm described above can determine the values of all the parameters in a system whenever there are more data points than unknowns. However, this is rarely achieved in practice. The main reason arises out of a phenomenon known as correlation. This essentially means that variations in certain parameters have similar effects on the objective function. So, error in one parameter can be compensated by error in another. Thus, the ability of the optimisation procedure to distinguish between correlated parameters is degraded. With perfect data (i.e. no experimental error) all the parameter values could be determined but as experimental noise in the data increases, the number of such parameters decreases. Systematic errors are

considerably more detrimental in this respect than random ones. This is because regression analysis assumes that all errors are normally distributed. Not all parameters are equal in this context: some pairs will be more highly correlated than others. The implication for experimental design is that only one parameter from a significantly correlated set can be determined by an optimisation procedure. Unfortunately, there is no sharp dividing line to indicate when an ill-conditioned system has been selected. This forms the crux of the discussion in Section 3.3.

3.2 Optimisation procedures for glass electrode calibration

The experimental determination of free hydrogen ion activity or concentration is perhaps the most frequently used of all analytical techniques. The convenience and suitability of glass electrodes for this purpose has promoted their application in a wide variety of contexts and this has consequently been responsible for a great deal of research into how they function. Nevertheless, accurate measurements using glass electrodes remain more elusive than is commonly supposed. Problems concerning the way in which the electrode system should be calibrated are probably the most worrying cause. They are crucial to the vast majority of potentiometric determinations of metal-ligand formation constants. Accordingly, they will be considered here only in the context of titrations in which strong alkali is introduced into solutions held at constant temperature and ionic strength.

Consider an electromotive cell in which a test solution surrounding a glass electrode is in electrical contact with a reference electrode via a salt bridge. It could be represented as follows:

first reference half-cell (in glass electrode)	test solution	second reference half-cell (external)
	g	l

The boundaries, g and l, respectively, indicate the glass membrane and the liquid junction at the interface between the salt bridge and the test solution. There are four contributions to the measurable potential difference between the two reference

electrodes [305]. Two arise from the reference electrodes themselves. They will have opposite signs and will usually be of comparable magnitudes. Most importantly, their contribution will be independent of the composition of the test solution and so may be represented as a fixed, combined potential, E_r. On the other hand, the potential differences generated across the boundaries of g and l will depend heavily on the activities of all the chemical species on either side of them. Such potentials arise from the disparate rates at which ions are transferred across a boundary or, in the case of the glass membrane, appear to be so transferred. Letting E_g and E_l represent these boundary potentials, the measured e.m.f. of the cell is thus given by equation 3.1.

$$E_{cell} = E_r + E_l + E_g \qquad (3.1)$$

In practice, it is found that the free hydrogen ion concentration is by far the most important component determining the values of E_g and E_l. This is because the glass electrode behaves as a selective cation exchanger for hydrogen ions [1942] and because the mobility of both hydrogen ions and hydroxide ions is so much greater than that of other species [1939]. In the case of the liquid junction, considerable changes in the composition of the test solution are required to significantly alter E_l. So, for the time being, this will be considered as constant. Quite the reverse is true of the glass membrane. It is very sensitive to changes in hydrogen ion activity and indeed most glass electrodes are found experimentally to exhibit a Nernstian response over a wide range of concentration [1974].

Accordingly, equation 3.1 can be re-written as equation 3.2.

$$E_{cell} = E_r + E_l + E_g^\circ + \frac{RT}{F} \ln \{H^+\} \qquad (3.2)$$

E_g° is the standard glass electrode potential at unit activity of hydrogen ions, R is the universal gas constant, T is the absolute temperature and F is the Faraday.

As long as the ionic strength of the test solution remains constant, the free hydrogen ion activity, {H}, can be expressed in terms of concentration. Hence, one obtains equation 3.3 by collecting together all the constants as E_{const} and putting $s = 2.303RT/F$.

$$E_{cell} = E_{const} + s \log[H^+] \qquad (3.3)$$

The above treatment specifically avoids referring to pH. Nowadays this quantity is defined by IUPAC as an empirical function of the difference in e.m.f values of the electrode between the test solution and one of a set of buffers prepared according to standard procecures [1458,1459,1479,1796,1947]. The obvious disadvantage with this approach is that it removes any direct relationship between pH as the experimental quantity and the actual free hydrogen ion concentration. By carefully selecting the buffer solutions which are prescribed as standards, significant discrepancies between pH and the activity of the hydrogen ion have been avoided. Nevertheless, three fundamental weaknesses exist.

1) In specifying the pH of a standard buffer, exact account must be taken of the effect of the various solute species on the

activity of the hydrogen ion and the degree of dissociation of the buffering species must be precisely known.

2) Even when the first point can be neglected, the pH of the test solution does not correspond to the hydrogen ion activity because neither the liquid junction potential, nor the activity coefficients will be the same in the test solution as in the buffer.

3) For practical purposes, when one wishes to know the free hydrogen ion concentration rather than its activity, an extended Debye-Hückel type correction must still be applied to measurements of pH made in the test solution.

Quantitatively, points 2 and 3 are the most significant and so a surfeit of methods have been developed to correct for them [62,291,292,324,361,450,1514,1796,1947,1974]. However, even these are obliged to assume that the activity of hydrogen ion in the buffer solution is given by 10^{-pH}. With the increasing precision of modern glass electrodes and potentiometers, this is no longer satisfactory [Jameson,R.F. - personal communication]. Indeed, inconsistencies between different buffers would appear were they not defined in such a way that their pH values are adjusted to conform with a single, primary standard.

These reservations concerning the use of buffers to calibrate glass electrodes are exacerbated when high concentrations of background electrolyte are used to maintain a constant ionic strength in the test solutions. Thus, researchers who employ potentiometric methods to measure formation constants have long

used solutions of known hydrogen ion concentration instead [367]. This is often done by titrating strong acid solutions with strong alkali and plotting the e.m.f. at each point against the corresponding values of $\log[H^+]$. The procedure should yield a straight line from which the intercept, E_{const}, in equation 3.3 can be obtained by extrapolation to $\log[H^+] = 0$.

There are several reasons why an ideal response is not observed in practice. Chiefly, one finds that unless there is a sufficient excess of acid or alkali to ensure that the solution is concentration buffered, small errors become very significant. It has, for example, been shown that the presence of glass itself causes a large deviation [395], a fact that can probably be attributed to hydrogen ion adsorption onto the glass surface. Another important factor is the imperfect behaviour of glass electrodes in alkaline solution: many of the types of glass used for electrode manufacture become increasingly sensitive to metal ions, especially sodium ions, above $-\log[H^+] = 11.0$ [655]. The effect of hydrogen ion concentrations on liquid junction potentials has already been mentioned.

Accordingly, there is a restricted range of free hydrogen ion concentration over which strong acid *versus* strong base titration data is suitable for calibration purposes. The most linear response occurs between $-\log[H^+]$ values of 2.3 and 2.9 [395]. Data collected inside these limits with most research equipment can yield E_{const} to a precision of about 0.1 mV [1974].

This sort of precision is more than adequate for the determination of formation constants for biological systems. What is less satisfactory, however, is that the critical measurements with most systems lie outside the calibration range. So hydrogen ion concentrations must be procured by extrapolation. Also, it is well known that the standard potential of the glass membrane varies from day to day (due to assymmetry effects) and that E_{const} may be affected by other changes (particularly if the liquid junction is not perfectly reproduced). These can be significant even from one experiment to another [655]. *Thus, internal calibrations of the electrode, performed in the test solution itself, are highly desirable.*

In principle, there are two main ways of achieving this goal. The first applies to solutions in which the introduction of strong acid is totally reflected as an increased free hydrogen ion concentration. It is then possible to calibrate by a series of constant additions. However, this cannot be done with weak acid or weak base solutions. Instead, one can calculate the free hydrogen ion at various points from the protonation constants, provided these are accurately known. Very precise calibrations can be made in this way but they are of limited use. Indeed, the object of many titrations is to measure the protonation constants. In these cases a number of parameters, namely, E_{const} and one or more equilibrium constants, must be determined simultaneously. Straightforward solutions are available only very rarely so general optimisation techniques must be employed.

3.2.1 Multiple analysis of titration data

The first extensive application of numerical methods to the treatment of titration data was published by Sillén and co-workers nearly two decades ago [15,16,18,44,45,46,47,397]. Since then, a large number of computer programs have been developed to refine formation constants. Some of these are discussed in Section 3.3 but here attention should be focused on the optimisation of other parameters, especially in the context of glass electrode calibration.

Sillén realised that the data collected during an average titration contained a great deal more information than was traditionally utilized. For example, small adjustments to the initial concentrations of components could significantly reduce the sum of squares objective function and so, conversely, this criterion could be used to improve the concentration values. Surprisingly, the idea does not seem to have been widely adopted, possibly because of the dangers discussed in the next section which are always inherent in optimisation procedures. Nevertheless, it seemed a potentially rewarding way to approach the problems of glass electrode calibration.

A computer program called MAGEC (for Multiple Analysis of titration data for Glass Electrode Calibration) was accordingly developed. The overall aim was to process data from any titration involving acids or bases by a variety of methods so as to maximize the information which could be obtained from it. Whilst the chief object was to evaluate the intercept in equation 3.3, it was also required that the value of any other

parameter of the system could be examined simultaneously and that the effect of errors in any estimated quantity could be determined. The FORTRAN listing for program MAGEC is supplied as Appendix A4. The preparation of input data is dealt with in Section 3.3 and in Appendix A5.

3.2.1.1 Analysis of strong acid *versus* strong base titrations.

MAGEC first analyses strong acid *versus* strong base titrations by the method of Gran [1613, 1614, 1770, 1771, 1780, 1781, 1782, 1783, 1784]. By transforming the potentiometric data into a linear form, this gives a good indication of glass electrode performance and also yields an endpoint that is independent of the slope and intercept used in Equation 3.3. Furthermore, if extrapolation of the data from before the endpoint produces a value significantly lower than that obtained from data after the endpoint, it suggests that an alkaline titrant may have become contaminated with carbon dioxide from the atmosphere [1770]. The endpoint obtained from the Gran extrapolations provides an independent check of the MAGEC optimisation of the strong acid concentration referred to below.

Further processing of strong acid *versus* strong base titrations is divided into three stages. To begin with the input concentrations are used to calculate free hydrogen ion concentrations at each point and a linear least squares fit is performed

on the data before and after the endpoint and over the entire range. This first analysis is used mainly for comparison with subsequent output. Due to relatively small errors in the concentrations of titrant and titrand, the least squares straight line does not normally possess a Nernstian slope. So the concentration of the titrand is varied slightly until the slope from the data before the endpoint coincides with the theoretical value. This optimisation yields an endpoint which has proved invariably to lie within the limits which can be determined from the Gran plot. If anything, the precision is somewhat better.

It is then possible to adjust the titrant concentration in a similar manner to that described above but on the basis of the whole range of data. Of course, to maintain the same endpoint, a corresponding change in the titrand concentration also needs to be made. In this way, very close agreements between the calculated and observed values for the e.m.f. at each point can be achieved. In acid solutions, there are often no residuals greater than the 0.1 mV which corresponds to the precision of many digital read-out research potentiometers.

The power of this analysis warrants some caution. Two factors critically affect the refinement of the titrant concentrations in the final stage. The first is the value of the dissociation constant of water. This parameter is very highly correlated with the concentration of alkali in the burette, i.e. a small error in its value will cause a significant deviation

in the apparent slope of the electrode response because it is used to convert hydroxide ion excess into free hydrogen ion concentrations and so it manifests itself in the optimised alkali concentration. Another way of looking at this is that either the titrant concentration or the dissociation constant (but notboth) can be determined by finding the value which yields a most ideal least squares slope. To accommodate those situations in which the dissociation constant of water is uncertain, MAGEC permits the user to vary the estimate systematically.

The second factor is fundamentally harder to overcome than the first. It is that glass electrodes in alkaline solutions tend to give less than an ideal response. There is thus a limit to the precision with which titrant concentrations can be determined by MAGEC; in practice, it is rather poorer than the accuracy with which the solutions can be prepared. This, in turn, suggests that it would be unwise at present to rely on a dissociation constant of water determined by potentiometric titration using glass electrodes. So the main value in analysing the alkaline data lies in the estimation of the errors which it affords and in the investigation of ways to improve the situation. For example, it is possible that a considerably improved optimisation procedure would be possible if the effect of sodium ion in these alkaline solutions was taken into account.

One valuable attribute of program MAGEC that was not fully appreciated until after the program had been developed, concerns the deterioration which all glass electrodes eventually undergo.

Lifetimes differ considerably and it is often difficult to detect the first signs of a failing performance. The MAGEC analysis of strong acid *versus* strong base titrations has proved very revealing in this regard: sudden and marked increases in the residuals occur just prior to the electrode's demise. Moreover, the development of discrepancies between the endpoint determined by MAGEC on the basis of a Nernstian response and that found by the Gran plot suggests that the electrode is no longer functioning ideally.

The usual procedure for analysing strong acid *versus* strong base titrations using program MAGEC is depicted in Figure 3.1. A typical result is shown in Example 3.1.

3.2.1.2 Analysis of titrations involving weak acids or bases.

In the case of weak monoprotic acids and bases, MAGEC utilizes a number of approximation formulae* [84,215] to solve for the free hydrogen ion concentration at each point in the titration. Analysis of the data can then follow a similar approach to that described above for strong acid and strong bases.

On the whole, however, this is not very satisfactory because it is much more difficult to know when the solution can be considered reasonably well buffered. A very sophisticated function for weighting the data is required to prevent unbuffered points from swamping the objective function and making it insensitive to an improved fit between calculated and observed points in the more important areas of the titration.

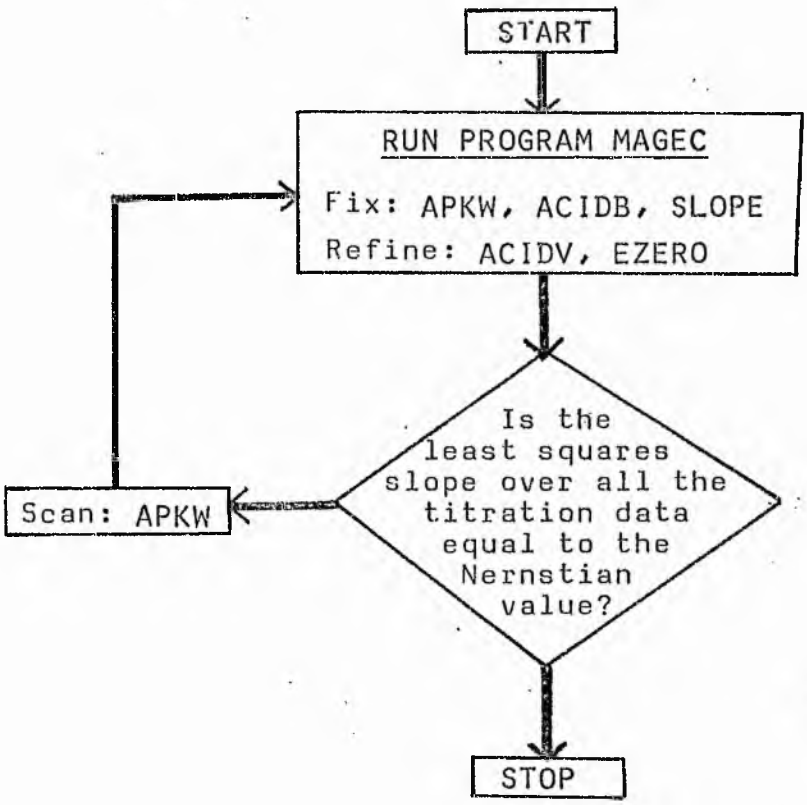
---

* For example, the Henderson-Hasselbalch equation:

$$-\log [H^+] = -\log K_a + \log \frac{[salt]}{[acid]}$$

FIGURE 3.1 USE OF PROGRAM MAGEC

Flow diagram of the usual procedure with strong acid *versus* strong base titrations



Symbols

APKW	=	apparent dissociation constant of water
ACIDV	=	titrand acid concentration (negative for alkali)
ACIDB	=	titrant acid concentration (negative for alkali)
EZERO	=	electrode intercept
SLOPE	=	electrode slope

EXAMPLE 3.1 USE OF PROGRAM MAGEC

Calibration of the glass electrode by strong acid *versus* strong base titration

A solution of hydrochloric acid (10.00 cm^3, *ca.* 50.00 mmol dm^{-3}) and sodium chloride (20.00 cm^3, 212.0 mmol dm^{-3}) was titrated with sodium hydroxide (100.0 mmol dm^{-3}) and sodium chloride (100.0 mmol dm^{-3}) solution. The sodium chloride was used to maintain the ionic strength (*ca.* 150 mmol dm^{-3}) as constant as possible. The titrated solution was thermostatted at 37.0 ± 0.1°C.

A Gran plot analysis for MAGEC gave endpoints of 4.977 ± 0.002 and 4.97 ± 0.03 cm^3 for the acid and alkaline data respectively. The MAGEC optimisation analysis is summarised in the following table.

		Before MAGEC optimisation	After optimisation of the acid concentration
Using only the data before the endpoint (47 points)			
(i)	electrode intercept	362.4 ± 0.15	360.7 ± 0.04
(ii)	electrode slope	62.48 ± 0.07	61.53 ± 0.02
(iii)	standard deviation	1.4×10^{-1}	4.1×10^{-2}
(iv)	number of residuals greater than 0.1 mV	26	0
Using all the buffered data (63 points)			
(i)	electrode intercept	360.4 ± 0.10	360.6 ± 0.05
(ii)	electrode slope	61.53 ± 0.02	61.50 ± 0.01
(iii)	standard deviation	5.0×10^{-1}	2.6×10^{-1}
(iv)	number of residuals greater than 0.1 mV	41	15

Note:

(i) The optimised initial acid concentration of 16.59 mmol dm^{-3} compares with an expected value of 16.67 mmol dm^{-3} and yields an endpoint of 4.978 cm^3.

(ii) A slope of 61.50 mV corresponds to pK_w = 13.310. In practice, the calculation could be repeated using pK_w = 13.305 to obtain a better agreement with the Nernstian value (61.54).

Some time was spent looking for a good weighting procedure but this was met with no success. The motivation for this came from a statement by Sillén which implied that titre volumes should be used as the independent variable in this sort of optimisation procedure [16]: free hydrogen ion concentrations should be calculated from titre volumes and thence converted into values of e.m.f. In spite of this, all the optimisation programs in this field work the other way around, taking e.m.f. as the independent variable and calculating titre volumes. Presumably the reasons are that this is mathematically simpler and automatically weights the data in favour of the most buffered points. (In unbuffered regions, errors in the e.m.f. values will have very small effects on the titre volume that is calculated.)

Accordingly, the main analysis applied by MAGEC to all titrations involving ligands is one of general optimisation of parameter values by minimising an objective function based on titre volumes. Any parameter can be flagged for refinement so specific procedure is left largely in the hands of the user. Usually, the requirement is to find the value for E_{const} in each of a series of titrations with the ultimate objective of determining the protonation constants of a ligand. Experience has shown that the best way to do this is in conjunction with another program that optimises protonation constants over the whole set of titrations (see Section 3.3). MAGEC deals with only one titration at a time so no distinction is made between local and global parameters. Hence, it is first used to estimate E_{const}

and the protonation constants applicable to each titration.

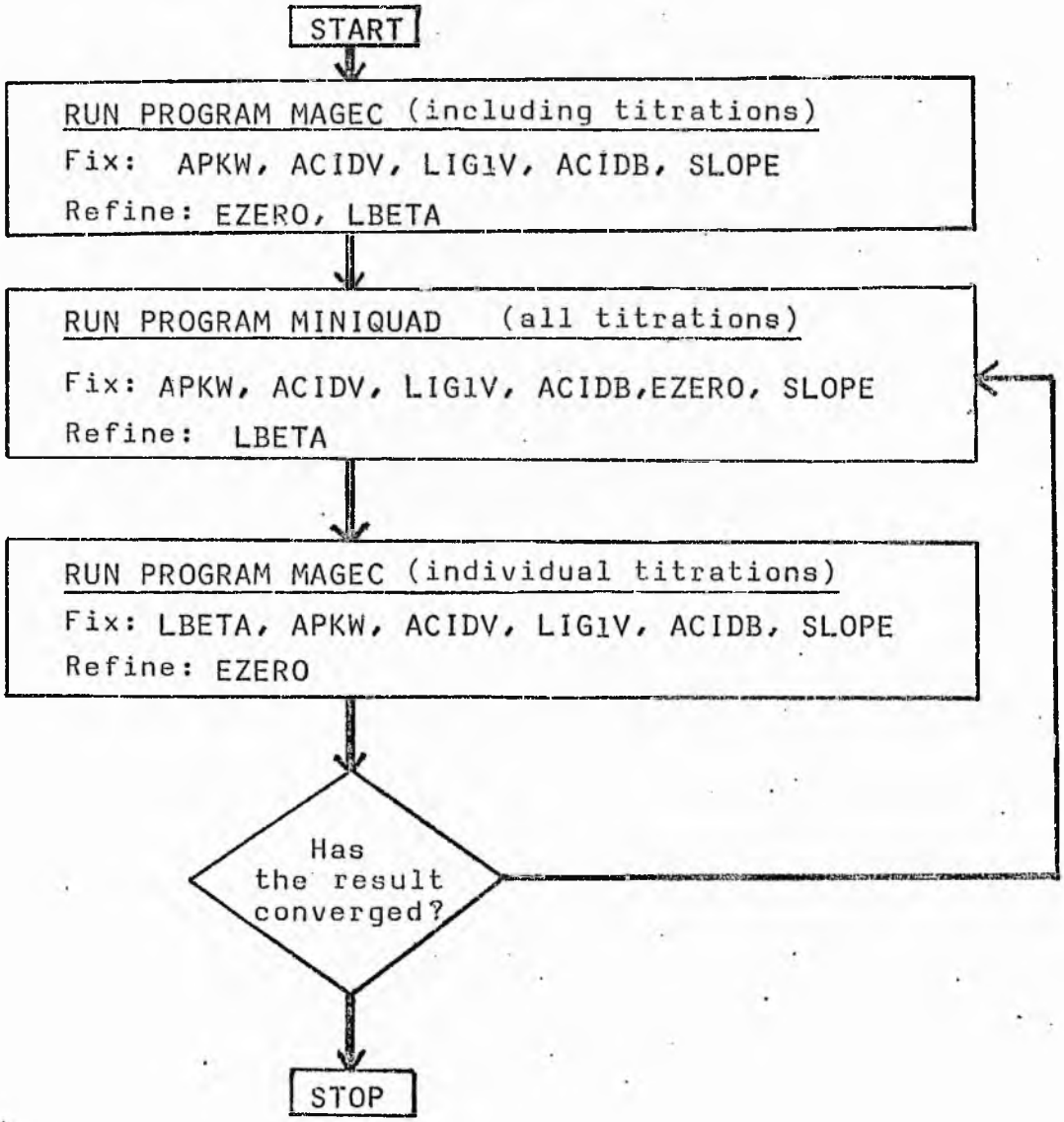
The constants so obtained will differ from one titration to another due to experimental error so they are averaged before being fed (together with the various values of E_{conet}) into the second program for global refinement. A cycle is thus initiated in which improved estimates of the protonation constants are held invariant by MAGEC to obtain new values of E_{const} and these are used in the other program to further optimise the protonation constants. Only one or two such iterations are usually required before convergence is reached. Substantially enhanced agreement between observed and calculated data can thus be achieved.

The above procedure is depicted diagrammatically in Figure 3.2. A typical result of using program MAGEC to optimise the electrode intercept in titrations involving ligands is shown in Example 3.2.

Alternatively, MAGEC may be used to check or adjust the concentrations of titrand or titrant. If the protonation constants are known this offers a very precise analytical tool. On the other hand, it is sometimes possible to refine concentrations as well as the protonation constants and E_{const}. Provided the dangers of correlation referred to in Section 3.1 are carefully avoided, the program therefore offers a very effective way of dealing with the many ligands which have not previously been studied under biological conditions of temperature and ionic strength.

FIGURE 3.2 USE OF PROGRAM MAGEC

Flow diagram of the usual procedure with titrations involving ligands



Symbols

LBETA	=	ligand protonation constants
APKW	=	apparent dissociation constant of water
ACIDV	=	titrand acid concentration (negative for alkali)
ACIDB	=	titrant acid concentration (negative for alkali)
LIG1V	=	ligand concentration in vessel
EZERO	=	electrode intercept
SLOPE	=	electrode slope

EXAMPLE 3.2 USE OF PROGRAM MAGEC

Internal calibration of the glass electrode in titrations of ligand solutions

A solution of glycine (20.00 cm^3, 10.12 mmol dm^{-3}) in hydrochloric acid (9.936 mmol dm^{-3}) was titrated with sodium hydroxide solution (100.0 mmol dm^{-3}). A background concentration of chloride ions (150.0 mmol dm^{-3}) was maintained and the solution was thermostatted at 37.0 $\pm$ 0.1°C. The assumed equilibrium constants were the ionic product for water (pK_w = 13.310) and the acid dissociation constants for glycine (pK_{a1} = 2.334 and pK_{a2} = 9.250). The electrode slope was considered to be Nernstian. The initial electrode intercept of 360.0 mV was refined to 363.8 mV in 7 iterations, improving the sum of squared residuals from 1.18 x 10^{-1} to 1.65 x 10^{-3}. The random distribution of the final residuals in the following table is noteworthy.

titre volume (cm^3)	observed emf (mV)	initial residuals (cm^3)	final residuals (cm^3)
0.00	220.95	-0.176	0.003
0.10	219.10	-0.173	-0.001
0.20	217.10	-0.167	-0.002
0.30	214.95	-0.159	-0.001
0.40	212.70	-0.150	0.001
0.50	210.30	-0.140	0.004
0.60	207.90	-0.135	0.002
0.70	205.30	-0.127	0.002
0.80	202.50	-0.118	0.003
0.90	199.60	-0.112	0.002
1.00	196.50	-0.106	-0.001
1.10	193.00	-0.096	0.001
1.20	189.15	-0.008	0.003
1.30	184.90	-0.075	0.004
1.40	180.20	-0.067	0.003
1.50	174.00	-0.046	0.013
1.60	168.00	-0.034	0.003
1.70	159.60	-0.022	0.004
1.80	148.00	-0.003	0.004
1.90	129.00	-0.015	0.002
2.00	44.00	-0.022	-0.003
2.10	-124.60	-0.028	-0.004
2.20	-145.80	-0.034	0.000
2.30	-158.50	-0.038	0.007
2.50	-167.95	-0.045	0.011
2.50	-175.75	-0.051	0.011
2.60	-182.25	-0.057	0.009
2.70	-188.10	-0.061	0.011
2.80	-193.70	-0.063	0.011
2.90	-198.95	-0.065	0.009
3.00	-203.95	-0.067	0.009
3.10	-208.95	-0.067	0.007
3.20	-213.90	-0.068	0.003
3.30	-218.85	-0.068	-0.001
3.50	-229.45	-0.068	-0.006
3.60	-235.00	-0.066	-0.008
3.70	-241.10	-0.066	-0.011
3.80	-247.50	-0.064	-0.012
3.90	-254.40	-0.064	-0.012
4.00	-261.55	-0.064	-0.012

The numerical method used by MAGEC was first published by Nelder and Mead [388] under the title "A simplex method for function minimisation". It was chosen for two main reasons 1) The objective function is coded in FORTRAN by the user as an external subroutine to the optimisation program. It is, therefore, very easy to modify the mathematical relationships being investigated. 2) The optimisation routine is set up such that the number and type of parameters being refined do not affect its structure in any way. So these can also be changed very readily. These advantages as well as the apparent robustness of the method, heavily outweigh the fact that simplex methods are not amongst the most efficient algorithms available nowadays.

Whilst work on MAGEC was in progress, another program with similar objectives appeared in the literature [1800]. Like MAGEC, this permits the optimisation of any or all titration parameters. However, it does not offer the other facilities simultaneously. Thus, the two programs partially overlap and partially complement one another.

3.3 Model selection

> Experiment is the interpreter of nature. Experiments never deceive. It is our judgement which sometimes deceives itself because it expects results which experiment refuses.
>
> Leonardo da Vinci
> 1452-1519

In one of the earliest reviews dealing with the measurement of formation constants in electrolyte solutions, Young and Jones commented that "we shall be fortunate if many more than half the values determined by methods such as these should eventually prove to be significant. Doubtless many complexes reported will be found not to exist in appreciable amounts and numerous others not reported will be shown to be important" [1986]. It is perhaps too soon to say whether their view was quantitatively pessimistic but it has proved completely justified in all other respects. The reason is not to be found in the experimental methods that have been employed but rather in the way the collected data has been evaluated [367]. Before the advent of generalised computer programs, researchers were forced to make simplifying assumptions about the equilibrium system that were not valid in many cases; since then, the position has been reversed and a multitude of spurious complex species have been diagnosed as a direct consequence of too much computational freedom.

The essential difficulty lies in model selection. Strictly a model is comprised of all the mathematical relationships required to describe an equilibrium system, including the parameter values. However, as everything else is usually well-defined, the term is often used less rigorously to mean the set of complexes present. Thus, the problem can be restated as the need to select those species which exist in the experimental solutions. Their identity is not generally known in advance and neither is it easily determined from the data.

Optimisation procedures inherently require that a model be specified *a priori*. It is used by the numerical technique to repeatedly simulate the experimental system using various sets of parameter estimates. In this way, the best fit between calculated and observed data is obtained. The important point is that for each model which might be chosen, the optimisation of a parameter such as a formation constant will converge on a different value. Naturally, the effect exerted by the parameter in question depends on its mathematical environment and this, in turn, is subject to the model. So unless the correct model is chosen, optimisation can be expected to yield incorrect values.

There are two complementary reasons why the correct model cannot automatically be selected by a numerical technique. Firstly, the correlation which can occur between different parameters (as discussed in Section 3.1) permits error in one parameter to compensate for error in another. Thus, multiple solutions may be found with similar values for the minimised objective function. Secondly, the effect of an incorrect model on the optimisation is identical to a systematic experimental error (*vide infra*) so whenever the errors in the data are not perfectly random and normally distributed, the uniqueness of the correct model is lost.

This has profound practical consequences for those who use computer optimisation methods for determining formation constants. With incorrect and sometimes even palpably unrealistic models one can obtain apparently satisfactory solutions. A common pitfall is to assume that the best model generates the

lowest sum of squared residuals. This is fatal when the models being compared differ in the number of parameters being refined. (The greater the number of degrees of freedom enjoyed by the optimisation procedure, the better the fit it should find.) Conversely, good agreement can be achieved without including all the complex species in the model that actually exist in solution. This usually implies that inadequate information has been obtained experimentally. In such situations, apparent success is even more readily forthcoming when an incorrect species with a similar effect on the objective function to the real one has been substituted in the model.

What can be done about these hazards? Certainly, wider recognition that there is no clearcut or guaranteed method for deciding on the most appropriate model would, in itself, reduce the number of erroneous formation constants appearing in the literature. Greater frankness about those circumstances when a scientific distinction between two or more models cannot be made would be encouraged. Thus, there would be fewer arbitrary or prejudicial choices for the sake of simplifying results. The words of Leonardo da Vinci cited at the beginning of this section might even prompt some investigators into clarifying the position by further experiment!

To the extent that there are no hard and fast rules about model selection, it may be regarded as the art of optimisation. This does not mean there are no guidelines or objective criteria, however. On the contrary, there are many. The difficulty is to decide on their respective importance.

Quantitatively, there are a number of statistical yard-sticks which are usually very helpful. When these are used, model selection becomes synonymous with statistical hypothesis testing. The merits of particular models are assessed by the overall standard deviation in titre, the standard deviations of the formation constants themselves and other quantities such as the "crystallographic R factor". These criteria could be relied upon exclusively, provided the differences between models remained statistically meaningful. Strictly, this requires that the experimental error is random, normally-distributed and wholly localised in the dependent variable. Moreover, the residuals must all be weighted according to the inherent accuracy of the experimental measurement [16,18]. Under these conditions, Vacca has said "the whole of the computer and graphical determinations of stability constants should be rejected as statistically unsound" [1954]. The implication is that models should not be eliminated on statistical grounds alone unless they are very markedly inferior. On the other hand, the statistical criteria by themselves can never provide assurance that a particular model is the correct one. They can thus be used in a preliminary analysis to reject the most unlikely possibilities but the final choice must almost invariably be made by other means.

In cases where there is little to choose between different models statistically, it becomes important to consider the degree to which trends in the calculated and observed data correspond [1632]. This is chiefly because an incorrect model

corresponds to a systematic error so random distributions of residuals mean an increased likelihood that a good model is being used. However, there is another important factor. The degree and nature of the correlation between parameters depends on the objective function used, i.e. on the way the calculated data is obtained and which variable is used to make the comparison. The different effects of choosing e.m.f. or titre volume as the independent variable were mentioned in Section 3.2.1. It is reasonable to assume that the correct model will duplicate observed trends most faithfully, no matter which way the data is processed or which objective function is employed. Graphical representation of the data facilitates this kind of evaluation and often makes it possible to distinguish between two or more models which are not statistically different.

There are also a variety of indicators which argue against models by casting doubt on a complex species they contain. Generally, artefacts of the optimisation procedure tend to be very sensitive to changes in the computational environment. Thus, species associated with formation constants having high standard deviations should always be treated with caution. Large variations in the formation constant value obtained when other species are included or removed from the model are a similar manifestation of uncertainty and should be viewed likewise. In a parallel vein, it is important to check that all postulated species exist in significant concentration over a reasonable range of the data. Otherwise, the species is, at best, close to the limit of detectability. Under these circumstances, it

looks as though it should be included in the model only because it increases the number of degrees of freedom available to the optimisation process. It is thus wise to exclude it from the analysis even if it definitely does exist in solution.

It should be evident that reliable model selection requires a great deal of computation. It is advisable to employ more than one optimisation program and it is essential to have a variety of simulation procedures at one's disposal. Data preparation for many programs can, however, be very wasteful of both human time and computer resources. Input needs to be punched in the particular format for each program although their data requirements are often similar if not identical. Accordingly, a computer program called FORMAT has been written to eliminate much of this tedium. It accepts data prepared in a standard way and writes it to an intermediate computer file in a manner specified by the user. The programs for which data can be so transformed are MINIQUAD [330], MINIQUAD 76A [776], SCOGS [113], PSEUDOPLOT [317], ZPLOT, COMICS [111], ECCLES, and MAGEC. A major feature of the program is that it performs a variety of preliminary checks on the data. Unlike the programs for which the data is being prepared, it attempts to continue processing even after input errors have been detected. In this way, all the obvious problems are diagnosed in a single execution of the program. By contrast, mistakes in a data deck are traditionally detected one at a time because each error causes program termination. Therefore, there need to be as many computer runs as there are errors before the program can execute successfully. The instructions for using program FORMAT and the FORTRAN listing appear in Appendix A5.

CHAPTER FOUR

COMPUTER SIMULATION OF BIOINORGANIC SYSTEMS

4.1 Transition metal ions in biological systems

The transition metals normally account for less than 10 g of a 70 kg person [294,534]. At first sight, 0.01 per cent may seem a paltry amount for which the term "trace elements" is indeed justified. This lack of abundance, however, belies their biological significance. They are amongst about 30 elements deprived of which, sooner or later, all mammals perish [95,1610]. In this respect, of the 30, none can be ranked least important!

With the exception of molybdenum, the essential transition elements are restricted to the first of their three rows in the periodic table. These metals have been favoured by natural selection because they are relatively abundant and, for the most part, bioavailable [2,72,301]. Two special chemical features, in particular, have been exploited by evolution. The first is their capacity to bond with a characteristic stereochemistry to electron donors such as oxygen, nitrogen and sulphur atoms. The second is their facile participation in electron transfer reactions. These properties suit cyclic biological processes and have thus led to the incorporation of transition elements in one third of all known enzymes [73].

Without exaggeration, it can be said that the crux of life lies in the ability of enzymes to perform and regulate the host of necessary reactions under amazingly mild conditions of temperature and pressure. This is achieved by utilising the three-dimensional structure of proteins to create sites within the enzyme that can become thermodynamically very reactive [73]. Enzymes in such a state are said to be entatic [649,913]. Metal ions help to invoke this condition in two main ways. By bonding

to certain groups on the protein they may force it to adopt a particular configuration. More directly, they are often located at the active site where they become endowed with unusual properties due to the number and heterogeneity of nearby donor atoms and to the changes which can be imposed on the hydrophilicity of their environment. These factors may jointly generate atypical bond lengths, distorted geometries and odd coordination numbers which make the intermediate activated complex very amenable to chemical change [545,609].

Herein lies the reason why such small amounts of the transition metal ions in biological systems are sufficient. Their function is catalytic [170,363,544,574,911,1291]. The ubiquitous metalloproteins are involved in bioenergetics (reversibly transporting both oxygen and electrons) and in cellular biosynthesis and degradation of many substances including carbohydrates, lipids, proteins and nucleic acids. A large part of bioinorganic chemistry is devoted to the study of these molecules but so far computers have rarely, if ever, impinged on these investigations. This chapter is mainly devoted to questions about how transition metal ions are assimilated or eliminated by biological systems and how they are manipulated *in vivo* prior to metalloprotein synthesis. In this area computer models of low-molecular-weight complexing equilibria have made a substantial contribution to bioinorganic chemistry.

Investigators have gone to considerable lengths to establish which elements are essential [293,301,371,529,610,819,835,978, 1650,1840]. This is usually done by rigorously excluding the element in question from the diet and environment of the

experimental animal, usually a rat. Special cages must be built, the air cleaned and the food synthesised from the purest chemicals to ensure that the very lowest levels of contamination are achieved. Sometimes it is even necessary to extend the experiment over two generations because the requirement is so meagre that residual amounts in any normally born-and-bred animal are sufficient for good health. Essentiality is demonstrated when the deteriorating condition of the subject is reversed solely by the addition of the element to its diet.

On the other hand, it is well known that many heavy metals are toxic [229,343,430,855,1478,1539,1703,1755,1756,1757,1760,1818, 1831,1881,1887,1891]. The current concern with pollution has focussed attention on elements such as cadmium, lead and mercury which can have serious and permanent ecological effects when they are disposed in industrial effluents. Emotions are even more strongly aroused by radioactive heavy metal pollutants such as plutonium. The poisonous nature of these metals is to a large extent connected with the biological role of the essential transition elements. With their many and powerful metal binding sites, enzymes tend to be readily inactivated by co-ordination to unnatural ions [170,363,544,574,911,1291]. Moreover, the multitude of donor groups that are an inherent part of proteins provide all biological systems with an aptitude for progressive accumulation of heavy metals from their surroundings.

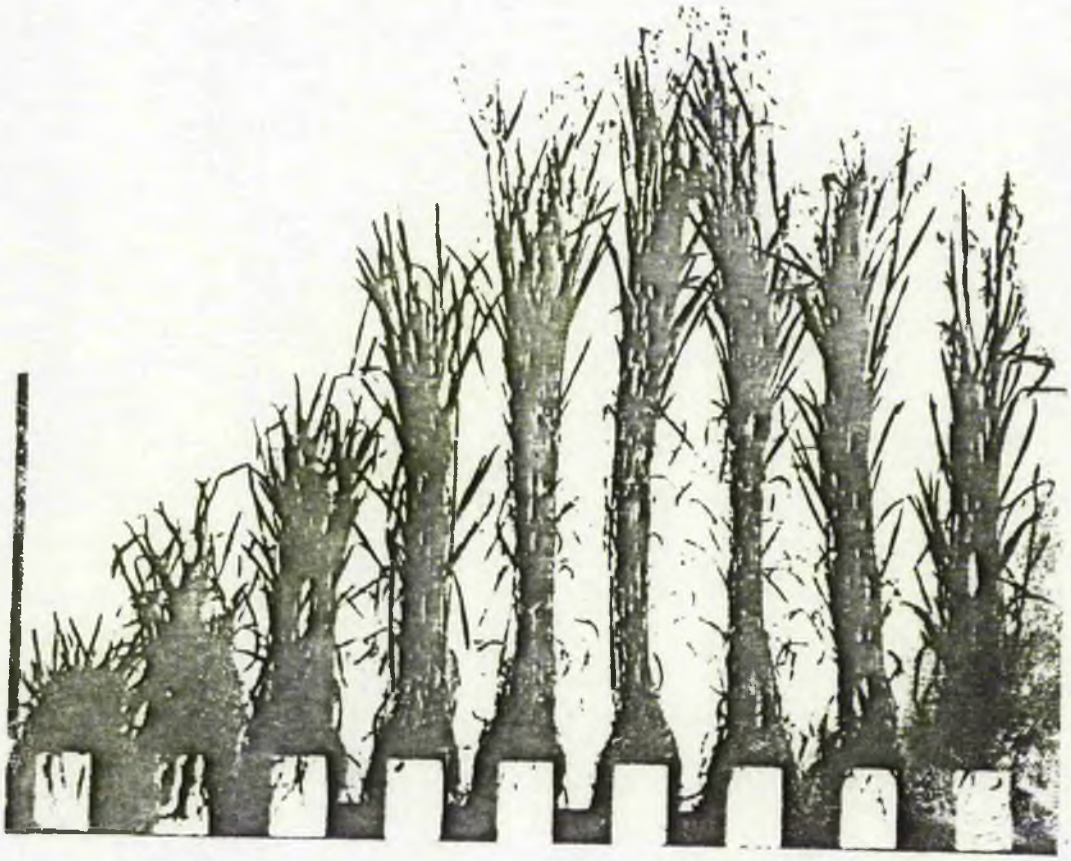
Although most first row transition metals are rigidly maintained in homeostatic balance, even the essential ones are toxic at high concentration so there is still the danger of

excessive assimilation. There is thus a critical balance between too much of each trace element and too little. This fact underlies almost all applications of bioinorganic chemistry to medicine. It is well illustrated in Figure 4.1 which shows the effect of various amounts of copper in the soil on the growth of oat seedlings - a marked optimum is exhibited below which malformation of the plants occurs because of an insufficient supply and above which they become increasingly poisoned. Whilst it does not involve a transition element, another striking example of this phenomenon is provided by arsenic. It has been proved recently that this traditional remedy for aggravating spouses is also an essential nutrient! [1650]

Certain medical implications of the aforegoing are obvious. Ways must be found to eliminate specific elements in those suffering from trace metal overload. Conversely, effective (but not excessive) supplementation regimens must be made available to those with trace element deficiencies. The health of many people could thus be radically improved. It has been said, for example, that in terms of the number of afflicted, iron deficiency anaemia is second only to protein malnutrition [405].

However, there are also more subtle medical effects which can be achieved by manipulating trace element concentrations *in vivo*. Many common diseases that do not stem directly from

FIGURE 4.1



Effect of copper on the height of oat-seedlings grown in nutrient copper-deficient medium. From left to right the quantities of copper present are nil, 3, 6, 10, 20, 100, 500, 2000, and 3000 µg per litre

metal ion overload or deficiency can be alleviated because either they, or immune responses towards them, depend on enzymes that are activated or inactivated by transition metal ions. Moreover, a number of transition metal ions participate in physiological processes such as DNA synthesis so they can interfere with cell replication. They are thus involved at a fundamental level in the physiology of the disease. For example, cancer cells are often associated with high concentrations of zinc [293] and consequently have a greater than normal requirement for exogenous supplies [638]. Accordingly, a great deal of research has gone into the relationship between metal, ligands and cancer [72,82,320,637] since Furst postulated a connection in 1963 [620]. There is even a commercially available anti-tumour agent called "Razoxane" that was originally synthesised in an attempt to produce an intracellular chelating agent [1808,1809,1810]. More recently, there has been a rapid growth of interest in the role of copper in rheumatoid arthritis. This subject is discussed in greater detail in Section 4.2.5.

4.1.1 The biological significance of the low-molecular-weight transition metal complexes

It is becoming established practice to classify transition metal species occurring in biological systems into four categories according to their molecular weight and the extent to which they are involved in the biofluid's labile metal ion equilibria [736,1250]. Figure 4.2 shows that there are generally three states between which metal ions can rapidly exchange: these are (i) certain metal protein complexes, (ii) some low-molecular-weight complexes,

FIGURE 4.2

Inert and labile metal-protein binding *in vivo*

Inert and/or thermodynamically Non-reversible	Labile and thermodynamically Reversible
IRON	
Haemoglobin	l.mol.wt. Transferrin $\rightleftharpoons Fe^{3+} \rightleftharpoons [Fe(H_2O)_6]^{3+}$ complexes
Myoglobin	
(Ferritin)	
COPPER	
Ceruloplasmin	l.mol.wt. Serum albumin $\rightleftharpoons Cu^{2+} \rightleftharpoons [Cu(H_2O_6)]^{2+}$ complexes
(Metallothionein)	
ZINC	
Alpha 2 macro	l.mol.wt. Serum albumin $\rightleftharpoons Zn^{2+} \rightleftharpoons [Zn(H_2O)_6]^{2+}$ complexes
-globulin	
(Metallothionein)	

and (iii) the aquated metal ions. The fourth type of species occurs when the metal is irreversibly bonded by a macromolecule and cannot be removed without disrupting the protein structure. The aquated metal ions have to be present for thermodynamic reasons but they usually occur at such low concentrations that their participation in any biological process is virtually precluded. At the other extreme, the high-molecular-weight species are at a kinetic disadvantage when the metal ion must diffuse through a biological membrane or be exchanged from one binding site to another. So, it is in these functions that the unique biological significance of the low-molecular-weight transition metal complexes is to be found.

In spite of their important role, the concentrations of the low-molecular-weight species must be kept very low in order to minimise the possibility of hydrolysis, polymerisation and precipitation [1631]. Moreover, the toxic effects of heavy metal overload are primarily manifestations of an enlargement of the low-molecular-weight fraction. To forestall such eventualities, carrier protein molecules with specific co-ordination sites have evolved. For the most part, the complexes they form are sufficiently stable and labile to ensure that all the other metal ion species present in the biofluid occur at very low but efficiently buffered levels.

There have, of course, been many attempts to assess the nature and magnitude of the low-molecular-weight fraction in biofluids such as blood plasma. Some of the first dealt with

copper [135,280] but since then others have encompassed iron [131,169,200] and zinc [140]. Such experiments have done a great deal to establish the classification shown in Figure 4.2. Indeed, the results of many earlier studies can now be considered as invalid because the equilibrium involving low-molecular-weight species was ignored [369]. Yet even today identification of the individual complexes present in the biofluid and determination of their concentrations remains fraught with difficulty. The concentrations are almost invariably well below the limits of analytical detection and techniques which alter them are likely to disturb the very distribution that one is attempting to monitor. Many common biochemical methods applied to bio-inorganic systems suffer from this defect (e.g. dialysis or column chromatography). So, more than ever, it is important to assess the effects of the analytical technique itself on the system being investigated. At the current state of the art, there is no reliable experimental method for looking at low-molecular-weight transition metal ion complexes in protein-containing biofluids. One is thus forced to rely heavily on the information provided by computer simulation models (Section 4.2).

A detailed discussion of the evidence for low-molecular-weight complexes influencing trace element metabolism is outside the scope of this thesis. There is such an abundance of fact that to deal thoroughly with it all would require a volume to itself. However, the essential features have often emerged from research into the physiology of iron. More work has been devoted to this trace element than to any other because of its essential

part in haemoglobin. Accordingly, the reader is referred to Appendix A6 in which the biological significance of low-molecular-weight iron(III) complexes is reviewed. It is necessary here only to mention two of the most important conclusions. The first is that, in the majority of cases at least, transport of transition metal ions through biological membranes occurs by passive diffusion. This implies that the lipophilicity and the charge of the predominant low-molecular-weight complex species formed *in vivo* will play a dominating role in determining the distribution of the metal ion amongst different body compartments [1075,1077]. The second conclusion concerns the equilibrium which is set up between high- and low-molecular-weight complexes in biofluids. There are a growing number of reports in the literature to suggest that a dynamic "steady state" relationship is physiologically very important [885,946,1060,1092]. In addition to the competition between low-molecular-weight chelating agents and labile metal protein complexes this would include the cycle of inert metalloprotein biosynthesis and degradation. By acting in concert, a very sensitive metal ion buffering system of considerable capacity could be achieved. The normal incorporation of iron into ferritin and of copper and zinc into metallothionein suggests that this is a fundamental aspect of the homeostatic regulation of many transition elements.

4.1.2 Bioinorganic drug design

Despite the medical rewards which adroit manipulation of metal ion concentrations *in vivo* could yield, progress in designing ligands for therapeutical purposes has been disappointing. In humans, even when the objective is simply to remove an offending metal ion or to supplement an essential one, the "treatments of choice" can rarely, if ever, be considered more than just satisfactory. There are many reasons for this. On the one hand, preventing excessive uptake of heavy metal ions is one of the vital functions of the intestinal mucosa. On the other, there are a number of inherent characteristics concerning the way in which chelating agents act in biological systems which militate against high therapeutic indices.

One aspect of the problem which is given almost universal cognisance concerns ligand selectivity and specificity. Clearly, it is desirable that the chelating agent binds the target metal ion strongly but leaves the multitude of other cations present in the body unmolested. Chemical principles for satisfying these criteria have long been established. One of the most obvious approaches to follow is Pearson's theory of Hard and Soft Acids and Bases (HSAB) [35,86,87,635,1426,1901] in which metal ions and ligands are classified according to their electrical polarizability and the most powerful interaction occurs between species with similar characteristics. In recent years, many agents have been produced which are extraordinarily specific and selective. Indeed, the contrast between this success and the

modest improvements achieved over the same period in chelation therapy itself have underlined the need to give more than the usual token consideration to the other factors which determine the overall biological response.

By far the most important aspect to which those researching into chelation therapy should pay greater attention concerns the multicompartmental nature of physiological systems. It is difficult to appreciate the full implications of this for optimal ligand design. As mentioned in Section 4.1.1, biological transport in this context is by passive diffusion so an ideal agent must satisfy at least three requirements in order to enhance urinary excretion of a toxic metal [1841]. It should (i) be sufficiently lipophilic to penetrate cell membranes in order to reach the sites of heavy metal deposition, (ii) form a lipophilic metal complex in the body compartment where the metal has accumulated and (iii) change to a hydrophilic complex in plasma so that a concentration gradient is established and the metal can be eliminated via the urine and not re-distributed into other tissues. These are demanding conditions which cannot easily be met. They imply that changes in lipophilic profile must be brought about by varying the electrical charge on the species, something which can only be accomplished if pH and other compositional considerations offset the increase in charge that naturally occurs with complex formation. Moreover, when the complex is to be excreted into the bile or when the drug is not administered directly into the bloodstream the above-mentioned requirements need to be modified and this means they almost invariably become more stringent.

Single ligands are thus, intrinsically, at a disadvantage. Many, like the polyaminocarboxylic acids, are almost entirely confined to extracellular space [1214,1805,1849]. They are thus forced to rely on a supply of the toxic metal from naturally occurring ligands that can mediate in the metal transfer between body compartments. This is, of course, always a relatively inefficient process but it is a particular handicap for exogenous ligands which have short biological half-lives in plasma. The disadvantage with making ligands more lipophilic is that this trend almost always parallels increasing toxicity [1319,1174,1175]. Moreover, as has been noted above, there is also the risk that lipophilic complexes can mobilise metal deposits and transfer them to less propitious sites.

For these reasons, the concept of "Synergistic Chelation Therapy" is proposed [1841]. It means the use of two drugs to collectively act upon the metabolism of the metal to promote its excretion. The simplest example occurs when one agent mobilises the metal in tissues while the other binds it in plasma for urinary excretion [1601]. This may involve the intracellular formation of a lipophilic complex that is dissociated by a more powerful hydrophilic chelator in plasma. Another illustration which may well prove effective in the treatment of siderosis would be the use of a relatively unsophisticated ligand to speed up the exchange of iron from transferrin to powerful chelating agents such as desferrioxamine [1525,1737].

Mobilisation, however, need not always depend on chelation *per se* [1602]. In the case of transition metal ions with two oxidation states that can co-exist *in vivo*, redox reactions can

rapidly release the metal co-ordinated within a protein matrix. A change in oxidation state will usually destroy the chemical and stereochemical compatibility between the binding site and the ion. This is relevant when the protein holds the metal in a kinetically inert manner provided there is sufficient equilibrium competition to remove the metal once lability has been established. It should be emphasised that redoxing agents must act within the context of the multicomponent equilibrium systems that operate in biofluids so, in contrast to the pronounced effect which can be displayed *in vitro*, their impact on the metabolism of metal ions such as iron and copper will be greatly moderated by biological buffering.

It is always tempting for those in search of better chelating therapeuticals to try to exploit endogenous ligands. These are certainly less likely to introduce toxicological complications. Yet, in practice, endogenous ligands have not proved very successful [909]. There are a number of reasons for this but two are paramount: (i) *in vivo* concentrations tend to be well regulated and (ii) to have an effect, the administered ligand must compete with natural levels of an identical substance and is, relatively speaking, therefore at a disadvantage. These points are particularly worth noting in the context of Synergistic Chelation Therapy. They have pessimistic implications for chelation strategies based on ternary complex formation with one or more endogenous ligands, regardless of the extra-stability often exhibited by mixed ligand complexes [see, for example, 1742,1841,1842 and 1956].

Attention has so far been focussed on chelating strategies aimed primarily at enhancing urinary excretion of toxic metal ions. This is because almost all current medical chelating agents work this way [909,816]. Elimination via the bile is another obvious route. The ligands are required to be sufficiently lipophilic to participate in the enterohepatic circulation of the bile acids and not be removed by renal filtration [816]. It has already been mentioned that the danger with increasing lipophilicity is that the agent itself invariably tends to become more toxic. The dichotomy is not easily bypassed but surfactant-like molecules can be synthesised with a range of aliphatic side-chain lengths in an attempt to find a happy medium. Alternatively, chelating agents with structural features similar to those of the bile acids may be made so that they selectively enter the liver. The rationale behind the development of the antitumour drug "Razoxane" may also be applicable: this bisdioxopiperazine, a cyclic imide derivative of EDTA, is sufficiently lipophilic to passively diffuse into cells where it turns into a chelating species by hydrolytic ring cleavage [1808,1809,1810].

The aim of this section has been to stress that the design of chelating therapeuticals requires more than concern with good metal-ligand specificity. Although most of the chemical principles involved have been known for a long time, it seems that progress in bioinorganic drug design has been slow because it is difficult to bring all the relevant factors to bear simultaneously. The development of sophisticated computer simulation models and the potential which they have for improvement, encourage one to feel that this need not remain a drawback much longer.

4.2 Computer simulation of metal-ligand equilibria in biofluids

From a chemist's viewpoint it is surprising how often the concept of speciation seems to have been overlooked in the biological sciences. No doubt the reason lies in the complexity of biological systems and in the very small amounts of substance which are often under investigation. In many circumstances it is difficult enough to keep track of the total concentrations, let alone worry about the stoichiometry and abundance of individual chemical species. However, this suggests that considerable advances in our knowledge of Nature can be made if and when the identification of the actual molecules involved in biochemical reactions becomes possible. This is particularly true in the bioinorganic context where the co-ordination partners of transition metals are very instrumental in determining how the ion behaves *in vivo* (see, for example, Appendix A6).

There are a large number of biofluids where information about the speciation of metal complexes would be of great interest. Some examples are seawater, ground water, blood plasma, intestinal fluid, and cell cytoplasm. This has encouraged a large number of investigators to develop computer models for the purpose of calculating various equilibrium distributions. The first was a computer simulation of seawater by Sillén [519,801]. This was met with the most remarkable success. It underlines the deep insight which models can provide. Sillén showed that pH buffering in the sea is primarily maintained by aluminosilicate clays and micas. This completely overturned the assumption that carbonate

equilibria were responsible. The computer models revealed that, contrary to wide belief, the ratio of carbonate to bicarbonate merely reflected the prevailing pH of the solution and did not control it except perhaps locally [653]. Since then several models of other natural waters have been published [244,246,535].

Blood plasma has also been the subject of many computer simulation studies. De Haven and De Land, for instance, have covered many aspects of blood biochemistry in their pioneering development of thermodynamic models for living systems [220,281, 378,380,381]. However, Perrin was the first to consider the distribution of transition elements amongst ligands in blood-plasma [3]. In a series of publications, the original model dealing with seventeen naturally-occurring amino acids interacting with Cu(II) and Zn(II) [158,286] has been extended to include Ca(II) and Mg(II) and a number of additional ligands [369,1500]. A detailed consideration of the merits of each model was included in this author's M.Sc thesis [736] and need not be reiterated here. Suffice it to say that the failure to take account of metal-protein binding in the biofluid was a major defect in the earlier work. In spite of attempts to improve the simulations by including protein species, this remains the issue most sensitive to criticism. The reason is that the formation constants of these species cannot yet be satisfactorily characterised in a thermodynamic sense. There are presently insurmountable difficulties in defining the exact stoichiometry of metal protein complexes, particularly with respect to the degree of protonation.

It is possible with well-behaved systems under specified circumstances to measure "conditional constants" instead. These partially describe the metal binding properties of the protein and applied with caution can provide valuable information about the distribution of metal ions in many biofluids. It is interesting to note how Perrin's results have evolved as his approach towards protein binding became increasingly sophisticated [c.f. 369 and 1500].

The computer investigations described in the remainder of this section yield, for the most part, relative rather than absolute results. This is largely because of the uncertainties associated with metal-protein binding. Thus, computer simulation techniques have been developed which bypass these difficulties by considering the *in vivo* complexing ability of chelating agents relative to one another. Very often bioinorganic interest is concerned with the respective effects of a series of ligands. In these cases, the additional confidence which can be placed in answers that do not depend on the exact extent of metal-protein binding outweighs the disadvantages of not knowing the absolute concentrations which are involved.

4.2.1 Metal-ligand complex distributions in normal blood plasma

There are two important reversible metal binding proteins in blood plasma. These are serum albumin and transferrin. A great deal of work has been done to investigate their interactions both with metal ions [29,30,31,151,160,168,185,190,269,350, 363,366,658,671,810] and low-molecular-weight anions [165,238, 247,248,290,556,1249]. Although some controversy surrounds the transport of labile Zn(II) [1186], this is in the face of overwhelming evidence that all the essential transition metal ions except Fe(III) are predominantly associated with the former protein [133,140,257,280,348,363,462,554,557,575,646,810,813,970, 1216,1247]. The same can be said of Ca(II) [1194,1527], Mg(II) [143,194], Pb(II) [28,225] and Ni(II) [910,974]. A specific binding site for Cu(II) exists at the amino-terminal end of the serum albumin molecule [178,180,181,739,740,1468]. On the other hand, transferrin clearly evolved to satisfy the special demands of iron transport *in vivo* [99,129,131,134,169,179,200, 353,433,483,560,589,991,992,1555,1556,1737] but it also binds, more powerfully than serum albumin, a variety of elements such as cobalt, chromium and plutonium [184,190,1164,1450,1451,1826].

This knowledge of the proteins to which metal ions in plasma are almost exclusively attached is fundamental to the calculations of the complex distributions of the transition elements between the low-molecular-weight ligands in normal plasma. Except for Ca(II) and Mg(II), [136,202], the free ion concentrations of the complexing metals can be anticipated to occur below the

limits which can be detected by ion selective electrodes even if they were present in pure water. The multitude and diversity of components in plasma make the analysis orders of magnitude more difficult so it is extremely unlikely that direct experimental determinations will become possible in the foreseeable future. However, the desired concentrations can be estimated from the "conditional" dissociation constants of the relevant metal protein complex by assuming that the vast proportion of the metal in plasma which is exchangeable is bound to the protein and that this quantity is negligible compared with the total protein concentration [736]. The best values currently available are shown in Table 4.1.

To compensate for the lack of analytical information about the transition metal ion concentrations in plasma, there is a wealth of data about the levels of low-molecular-weight ligands. Apart from a few such as salicylate and tryptophanate (where association with proteins must be taken into account (247,556)], the ligand concentrations available for complexing metal ions can now be obtained routinely by column liquid chromatography. Representative values are listed in Table 4.2.

The concentrations appearing in Tables 4.1 and 4.2 define a multicomponent equilibrium mixture whose species distribution can be obtained by computer simulation if all the formation constants for the various equilibrium reactions are known. How the latter values may be acquired has been described in Section 2.1.3. The outcome of the latest calculations for the

TABLE 4.1

Free concentrations of metal ions in normal blood plasma used in the simulations

Metal ion	Concentration (mol dm^{-3})
Ca^{2+}	1.13×10^{-3}
Mg^{2+}	5.2×10^{-4}
Zn^{2+}	1.0×10^{-9}
Mn^{2+}	1.0×10^{-12}
Cu^{2+}	1.0×10^{-18}
Fe^{3+}	1.0×10^{-23}
Pb^{2+}	1.0×10^{-14}

Values based on those given in [736] and [1250]

Ca^{2+} and Mg^{2+} accurate to ±5 %

Others accurate to an order of magnitude.

TABLE 4.2

Total ligand concentrations in human blood plasma, accurate to ±5% used in the computer simulations. All values lie within the limits of physiological variation.

	mol dm^{-3}		mol dm^{-3}
Alanate	3.70×10^{-4}	Serinate	1.22×10^{-4}
Aminobutyrate	2.40×10^{-5}	Threoninate	1.50×10^{-4}
Arginate	9.50×10^{-5}	Tryptophanate	1.00×10^{-5}
Asparaginate	5.50×10^{-5}	Tyrosinate	5.80×10^{-5}
Aspartate	5.00×10^{-6}	Valinate	2.27×10^{-4}
Citrullinate	2.70×10^{-5}	Histamine	3.00×10^{-8}
Cysteinate	2.30×10^{-5}	Carbonate	2.45×10^{-2}
Cystinate	4.00×10^{-5}	Phosphate	3.81×10^{-4}
Glutaminate	5.21×10^{-4}	Silicate	1.38×10^{-4}
Glutamate	4.80×10^{-5}	Sulphate	2.11×10^{-4}
Glycinate	2.43×10^{-4}	Thiocyanate	1.40×10^{-5}
Histidinate	8.50×10^{-5}	Ammonia	2.40×10^{-5}
Hydroxyprolinate	7.00×10^{-6}	Citrate	1.13×10^{-4}
Isoleucinate	6.50×10^{-5}	Lactate	1.82×10^{-3}
Leucinate	1.24×10^{-4}	Malate	3.50×10^{-5}
Lysinate	1.78×10^{-4}	Oxalate	1.20×10^{-5}
Methionate	2.90×10^{-5}	Pyruvate	9.50×10^{-5}
Ornithinate	5.80×10^{-5}	Salicylate	5.00×10^{-6}
Phenylalanate	6.40×10^{-5}	Succinate	4.20×10^{-5}
Prolinate	2.11×10^{-4}	Ascorbate	4.30×10^{-5}

Values based on those given in [736] and [1250]

most predominant complexes of Ca(II), Mg(II), Cu(II), Zn(II), Fe(III), Mn(II), and Pb(II) is depicted in Table 4.3. The percentage distribution obtained in the original studies is also given for comparison.

The most striking difference which is apparent concerns the distribution of Zn(II). Whereas the ternary citrato-cysteinato-Zn(II) complex was originally found to account for over 40% of the metal ion in the low-molecular-weight fraction, it is now of insignificant importance. The formation constants in question were redetermined by Dr.G.Berthon in St.Andrews [1632] in accordance with the principle laid down in Section 2.1.3 because the literature source from which the values had been taken [337] was open to some question. Fears that the extraordinarily high value reported was merely an artefact of certain ambiguities in the way the citrate complexing equilibria were defined appear to have been justified. Substitution of the new set of formation constants in the model reduced the concentration of the ternary species by almost four orders of magnitude [1632].

As a result of a number of recently published studies the formation constants of many other ternary complexes have also been updated. In this respect, Cu(II) has received particular attention [1587,1588]. However, the effect on the computer simulations has been marginal. It seems that for Cu(II) and Zn(II), at least, the distributions shown in Table 4.3 will stand the test of time because the formation constants of all the predominant species have now been measured experimentally and values for many of them have been independently confirmed.

TABLE 4.3

Percentage distribution of Ca(II), Cu(II), Fe(III), Pb(II), Mg(II), Mn(II) and Zn(II) amongst low-molecular-weight ligands in human blood plasma as found by computer simulation
% distributions are independent of free metal ion concentrations to a precision of 1%

Complex species	Percentage of the total metal	
	Current models	Original model[736]
$[Ca(CO_3)H]^+$	8	9
$[Ca(CitO)]^-$	4	4
$[Ca(PO_4)]^-$	3	3
$[Ca(LactO)]^+$	3	3
$[Ca(CO_3)]$	2	2
$[Cu(CisO)(HisO)]^-$	19	21
$[Cu(CisO)(HisO)H]$	15	17
$[Cu(HisO)_2]$	11	11
[Cu(ThrO)(HisO)]	9	8
[Cu(SerO)(HisO)]	6	4
[Cu(GlyO)(HisO)]	6	3
$[Cu(LysO)(HisO)H]^+$	5	5
[Cu(ValO)(HisO)]	5	5
[Cu(AlaO)(HisO)]	4	4
[Cu(GlnO)(HisO)]	3	2
[Cu(PheO)(HisO)]	2	3
$[Fe(CitO)OH]^-$	55	99
$[Fe(CitO)(OH)_2]^{2-}$	44	-
$[Fe(CitO)(SalO)]^{2-}$	<1	<1

/contd

Table 4.3 (contd)

$[Pb(CysO)]$	86	80
$[Pb(CysO)_2H]^-$	6	2
$[Pb(CisO)H]^+$	4	5
$[Pb(CO_3)H]^+$	2	2
$[Mg(CO_3)H]^+$	5	6
$[Mg(CitO)]^-$	5	5
$[Mg(LactO)]^+$	3	2
$[Mg(CO_3)]$	2	2
$[Mn(CO_3)H]^+$	23	24
$[Mn(CitO)]^-$	10	10
$[Mn(CO_3)]$	2	2
$[Zn(CysO)_2]^{2-}$	36	19
$[Zn(CysO)(HisO)]^-$	24	12
$[Zn(HisO)]^+$	4	3
$[Zn(CysO)_2H]^-$	3	1

The ligands are represented by symbols as follows:

AlaO = alinate; CisO = cystinate; CitO = citrate;
CysO = cysteinate; GlnO = glutaminate; GlyO = glycinate;
HisO = histidinate; LactO = lactate; LysO = lysinate;
PheO = phenylalanate; SalO = salicylate; SerO = serinate;
ThrO = threonate; ValO = valinate.

Considerably more work is needed to achieve this same standard with the other transition elements. Fortunately, it is the physiology of Cu(II) and Zn(II) which provides much of the impetus for the computer simulation studies reported in this thesis. Moreover, the models serve to highlight those systems with the other kinds of metal ion whose solution chemistry should be most urgently studied.

The computed percentage distributions of the newly-included metal ions Ni(II), Fe(II) and Cu(I) in plasma are shown in Table 4.4. It is not possible to obtain estimates of the free metal ion concentrations in the same way as for the other transition metal elements. There is insufficient knowledge about the binding of Ni(II) in blood plasma. One might expect the value to lie somewhere between that for Zn(II) and that for Cu(II) on the basis of an intermediate binding to serum albumin. With iron and copper, the metal ion in the predominant metal-protein complex exists in the alternate, higher oxidation state so "conditional" dissociation constants would be inapplicable. Instead, the levels are governed by the redox potential of plasma. In principle it is possible to calculate values from the Nernst equation using the standard redox potential of the couple between the metal ions in their respective oxidation states. However, this is subject to considerable uncertainty because plasma might not be at redox equilibrium. The redox potential of the biofluid can be gauged from the ratio of ascorbate to dehydroascorbate [577] but the value so obtained (-12 mV [577]) is in disagreement with the observed distribution between cysteine and its oxidised counterpart cystine [666]. The matter is further confused since

TABLE 4.4

Percentage distribution of Cu(I), Fe(II) and Ni(II) amongst low-molecular-weight ligands in human blood plasma as found by computer simulation

% distributions are independent of the free metal ion concentrations to a precision of 1%

Complex Species	Percentage of the total metal
$[Cu(CysO)H]$	45
$[Cu(CysO)]^-$	36
$[Cu(CysO)_2H_2]^-$	18
$[Fe(CO_3)H]^+$	27
$[Fe(CO_3)]$	22
$[Fe(HisO)]^+$	11
$[Fe(CitO)]^-$	6
$[Ni(CysO)(HisO)]^-$	44
$[Ni(HisO)_2]$	37
$[Ni(CysO)_2]^{2-}$	8
$[Ni(HisO)]^+$	3

The ligands are represented by the symbols defined in Table 4.3.

exact, absolute values for the standard redox potentials of sulphydryl compounds are not yet known because such systems tend to poison platinum and other noble metal electrodes [1984]. Nevertheless, assuming that the redox potential of plasma is about zero volts, one finds the approximate concentrations for Fe(II) and Cu(I) corresponding to the free concentrations of Fe(III) and Cu(II) shown in Table 4.1 are 10^{-11} mol dm^{-3} and 10^{-14} mol dm^{-3} respectively.

When the free concentrations of the transition metal ions in the computer simulations are varied to ascertain the effects of possible error, a most striking fact becomes apparent. Unless the values are raised very considerably, the percentage distributions shown to a precision of 1% in Tables 4.3 and 4.4 remain constant. In other words, the percentage of a transition metal in the low-molecular-weight fraction of blood plasma attached to specific ligands is independent of the exact free metal ion concentration and can thus be calculated without prior knowledge of the metal-protein binding constants [1234] or of the relevant redox potentials. This is so because the amount of metal complex formed in plasma is negligible compared with the amount of each ligand that is present. Complex species thus have no significant effect on the mass balance relationships of each ligand and consequently, the free ligand concentrations are not significantly affected by the degree of complexation. Under these circumstances, the concentration of each complex depends solely on the free concentration of the metal ion. In fact, there is a direct proportionality between them. As this is true for

all mononuclear species, the total concentration of each metal in the low-molecular-weight fraction is also directly proportional to the concentration of the free metal ion. Hence, the percentage of metal appearing in a given species is constant. Note that the information is relative. The absolute concentration of the free metal ions and of all their complex species are determined by the protein binding equilibria.

It should be stressed at this stage that computer simulations which yield relative answers are often very easy to misinterpret. More than usual care must be taken to avoid pitfalls occurring because only one part of the system is being modelled. This is well illustrated by the series of calculations reported in the earlier work [736,1250] which were made to determine the effect of an error in pH on the calculated percentage distributions. As expected, the simulations showed that the extent of metal complexation tends to increase as the free hydrogen ion concentration is reduced. For example, the calculated total concentration of the copper(II) complexes rose from 6×10^{-12} mol dm^{-3} at $-\log[H^+] = 7.2$ to 4×10^{-11} mol dm^{-3} at $-\log[H^+] = 7.6$. This does not mean, however, that the low-molecular-weight fraction will really be enlarged. On the contrary, as binding by proteins is also enhanced, the reverse is more than likely. The true effect of varying pH will only be seen in models which include formation constants for the metal-protein complexes that are correctly defined in terms of the free hydrogen ion concentration.

4.2.2 The effects of exogenous chelating agents on the metal-ligand equilibria in blood plasma

The most serious disadvantage with simulations yielding relative rather than absolute answers is that useful or even valid comparisons between models often prove difficult to make. This is the case when chelating agents are introduced into blood plasma. The obvious solution is to simulate treatment by including the administered drug at some realistic concentration in the normal model amongst the naturally-occurring ligands. However, the results of earlier work by this author [736] and by Perrin [369,1500] showed that such an approach was not ideal. The method makes assumptions about the way in which metal-protein complexes respond towards increased competition for metal ions. Although these were not fully considered at the time, it transpires that they are largely justified. Nevertheless, it is hard to perceive how the situation changes when the assumptions begin to fail. Moreover, the approach is not well suited to making predictions about the biological effects of a series of chelating agents. For example, one might like to compare the ability of various compounds to promote trace element excretion. There are two main reasons why a straightforward approach is awkward. First, one must choose a particular concentration in plasma that is applicable to all the agents but sufficiently high to reveal their important characteristics. Second, many chelators, even at relatively low concentrations, complex almost all of the metal ions in the low-molecular-weight fraction. Yet it is evident that different agents have different bioinorganic

properties in spite of the fact that many of them may have captured more than 99% of a given metal ion attached to low-molecular-weight ligands. Obviously, one reason for this is that they are also competing for the metal ions with plasma proteins. Their biological effect is manifest partly because they enlarge the low-molecular-weight fraction.

For these reasons, an extension to the procedure adopted for simulating the low-molecular-weight equilibria in blood plasma is proposed [1601]. The relative ability of an administered agent to compete for metal ions in the biofluid can be obtained by calculating the increase in size of the metal's low-molecular-weight fraction at any given ligand concentration. This factor, defined in equation 4.1, is called a "Plasma Mobilizing Index" (P.M.I.).

$$\text{P.M.I.} = \frac{\text{total concentration of all the metals' low-molecular-weight complexes in the presence of the drug}}{\text{total concentration of all the metals' low-molecular-weight complexes in normal plasma}} \qquad (4.1)$$

Consider the effect of increasing the concentration of a chelating agent in blood plasma. As long as the metal complexes it forms remain at concentrations very much lower than the metal protein complexes, the latter will continue to buffer the free metal ion concentrations at the level normally pertaining in the biofluid. Thus, the distribution of the low-molecular-weight

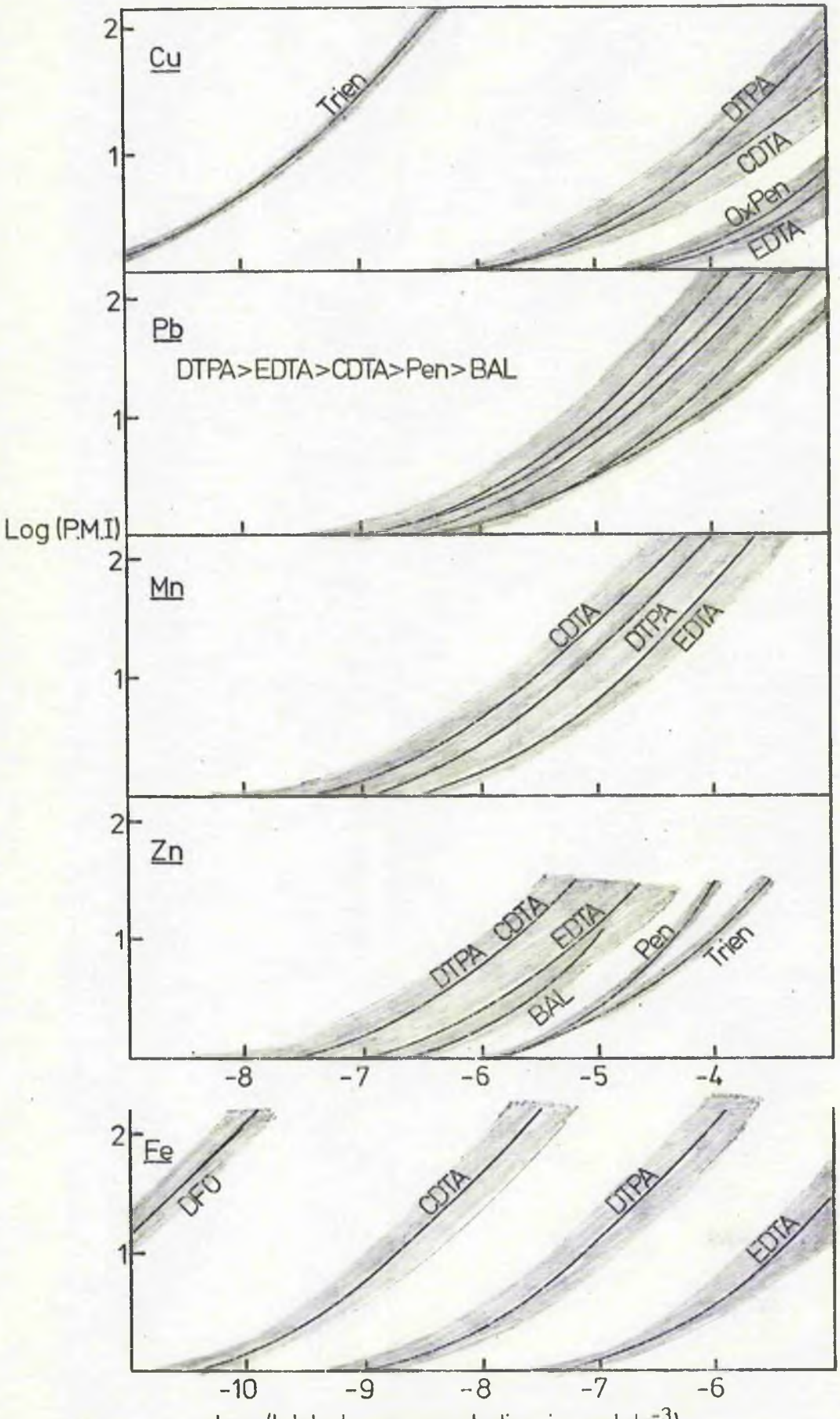
complexes changes because the administered chelating agent gains transition metal ions at the expense of the proteins and not the normal low-molecular-weight complexes. This situation changes only when the concentration of the metal-protein complexes begins to be significantly depleted. Whereupon the free metal ion concentrations will also begin to fall. Before this happens, however, the low-molecular-weight fraction of most transition metal ions will have had to have been increased by many orders of magnitude. Many interesting physiological effects may be expected solely from this enlargement.

Without characterizing the metal-protein binding, it is not possible to calculate P.M.I. curves over the whole range of chelating agent concentrations in plasma. Nevertheless, it is evident from equation 4.1 that the free metal ion concentration is a factor in every term in both the numerator and the denominator of the P.M.I. quotient. As only mononuclear complexes are found in plasma (because of the relative abundance of ligands over transition metal ions), it is thus possible to cancel the free metal ion concentration as long as it remains buffered by the metal-protein complexes. Under these circumstances, P.M.I. values become a relative measure of the metal ion binding ability of different chelating agents administered into plasma. Curves can thus be constructed to depict how the low-molecular-weight fraction is enlarged as the concentration of the chelating agent is increased [1600,1601].

Some examples where sufficient biological data exists to make for a worthwhile comparison are shown in Figure 4.3. The P.M.I. curves are calculated for (i) ethylenediaminetetraacetate (EDTA), (ii) diethylenetriaminepentaacetate (DTPA), (iii) cyclohexylenedinitrilotetraacetate (CDTA), (iv) desferroxamine (DFO), (v) penicillamine (Pen), (vi) 2,3-dimercaptopropanol (BAL), and (vii) triethylenetetramine (Trien). Their order correlates strikingly well with the ability of these agents to promote trace-metal excretion. Although direct comparisons between agents are not often made in the literature, their relative physiological efficacies appear to be as follows. For copper, Trien > DTPA > EDTA [944,1085]; for iron, DFO > CDTA > DTPA > EDTA > no effect [1030]; for manganese, CDTA = DTPA > EDTA > Pen = DFO = no effect [1046,1099]; for lead, DTPA > EDTA > CDTA > Pen = BAL> DFO = no effect [834, 1047,1169,1267]; for zinc, DTPA, CDTA > EDTA > Pen > DFO = no effect [1096,1112,1121,1151,1343]. Chelation therapy for nickel has so far only been studied in terms of the ability of the various agents to prevent mortality in acutely poisoned rats [1004,1071]. In good agreement with these observations, the computer simulations show that Trien and Pen are almost equally effective at binding exchangeable nickel ions in plasma and considerably better at this than the other agents investigated.

The good correlation between the calculated and observed data is particularly significant when one considers the major differences in biological response towards the chelating agents rather than their exact positions in the comparitive series given above. In this regard, the P.M.I. values, unlike the formation

FIGURE 4.3



(Shading indicates confidence placed in the respective formation constants)

constants, accurately portray the physiological properties. For example, if the excretion of lead and zinc, caused by DTPA, EDTA and Pen is considered, both the similarity of these ligands in their effect on lead [834,1047,1267] and the systematic differences they display towards zinc [1096,1112,1121, 1151] are reflected in Figure 4.3.

The P.M.I. curves for zinc in Figure 4.3 have been deliberately shortened. The reason is that buffering by metal-protein complexes is likely to fall off with this metal ion before it does so with any other. The total labile zinc concentration in plasma is about 10^{-5} mol dm^{-3}, some two-thirds of the total concentration [196,345,577,606,669]. From the free metal ion concentration shown in Table 4.1, the total zinc in the low-molecular-weight fraction will account for approximately 10^{-7} mol dm^{-3} [140]. Thus, the log P.M.I. values shown in Figure 4.3 are subject to a limit of about 2 units. Accordingly, the curves must taper off sigmoidally towards higher drug concentrations and would do so if the metal-protein complex equilibria could be adequately represented in the simulations. This phenomenon explains two observed characteristics of DTPA therapy which are otherwise difficult to comprehend [1646]. The P.M.I. curves suggest that at high DTPA concentrations, the ligand will be unable to obtain sufficient labile zinc from plasma proteins so there will be a significant fall in the free zinc ion concentration and a consequent increased binding of calcium ions. This shift in the equilibrium position would cause a transfer of zinc from tissues into plasma but this process

will probably be a relatively slow one. Thus, a dose of Ca-DTPA given in five fractions produces almost twice the urinary excretion of zinc than does a single equimolar dose [1078]. Moreover, the hypothesis also accounts for the enhanced removal of toxic trace metals by Ca-DTPA compared with Zn-DTPA [1078]: administration of the former produces a higher free ligand concentration because overall complexation is to some extent limited.

Whilst other contributions to the pharmacokinetics of the metal ion complexes formed *in vivo* can obviously be anticipated, the computer models show that, in most cases, the degree to which the agent competes for exchangeable protein-bound metal ions determines how effectively urinary excretion of the element is promoted.

Most of the agents considered in Figure 4.3 form highly charged complexes in blood plasma. They are thus confined to this body compartment until they are eliminated by the kidneys into the urine. Neutral complexes on the other hand may diffuse through membranes (Section 4.1.1). The models are thus able to explain the medical observation that plumbism is best treated in two stages - initial EDTA intravenous infusions are followed by longer term oral therapy with Pen [1118]. The negatively charged Pb-EDTA complex is strongly formed in plasma and quickly removed. However, lead which has dispersed into the tissues is inefficiently leached back into plasma by the polyaminocarboxylic acid. Pen forms a neutral complex which easily returns to plasma

once a concentration gradient is established. This also explains the experimental finding that premature treatment with Pen can be detrimental because it actually increases lead deposition in essential organs [1058]. Moreover, the serious side-effects caused by Pen-therapy can partly be attributed to zinc deficiency [1048,1343]. With zinc a negatively charged bis-complex predominates that is prone to urinary excretion [1601].

4.2.3 Computer simulation of Total Parenteral Nutrition

Recently there has been considerable progress made in the treatment of gastrointestinal diseases by the introduction of long-term intravenous feeding regimens [1976]. The technique, described as Total Parenteral Nutrition (TPN), employs solutions with compositions that have been established as suitable for maintaining healthy tissue metabolism and nitrogen balance in patients on shorter periods of supplementation. However, the need to extend the duration of this kind of therapy (often to several months and sometimes even to years) raises serious questions about the levels of micronutrients which should be administered simultaneously. A major concern lies in the inadequate supply of trace metals. If these are not included in TPN mixtures, a variety of protein and collagen biochemical processes are soon impaired and this is then followed by the development of overt deficiency symptoms [1873,1877,1977,1978,1979].

Zinc is particularly important in this context because (i) it has a major function in tissue repair [1235,1885], (ii) its urinary excretion tends to be increased after surgery [1983] and (iii) urinary losses are further promoted by the TPN infusions themselves, more so in fact than occurs with any of the other transition elements (*vide infra*).

Some clinical attempts to supplement trace metal concentrations in nutritive mixtures have been made [1873,1877,1879] but doses appear to have been chosen somewhat randomly and very little correlation between administered levels, plasma concentrations and overall metal ion balance was observed [1879]. It has been suggested that supplying zinc in excess of the measurable losses would satisfy the body's requirements [1981]. Such an approach remains empirical and difficult to generalize as this amount will vary from one patient to another. Furthermore, the relationship between the nutritive requirement for a metal ion and its rate of excretion is obscured by the nutritive treatment itself, as discussed below. It was, therefore, decided to investigate the effect of TPN mixtures on the metal-ligand equilibria in blood plasma to identify the most important interactions and if possible, suggest ways of estimating the specific doses of trace elements that ought to be included in future TPN preparations.

It is well documented that the infusion of naturally occurring ligands into plasma can cause a prompt and pronounced increase in the urinary excretion of trace elements, especially zinc. For example, intravenous administration of 250 $\mu g.hr^{-1}$

of histidine for three successive hours gave rise to zinc excretion rates of 7.6, 20.2 and 16.8 $\mu g.hr^{-1}$ compared with 0.4 $\mu g.hr^{-1}$ beforehand [1880]. This is in line with the fact that oral histidine greatly increases zinc concentrations in urine as has been shown with rats [857], dogs [1871] and man [1982]. Other natural chelating agents can produce the same sort of response: high urinary zinc losses are also introduced by cysteine [1871] and certain sugar-amine compounds [1746] but not by glycine [1871].

To identify the most predominant effects of the TPN infusions on the metal-ligand equilibria in blood plasma it was decided to imitate the infusion process by calculating the changes in amino acid concentrations that occur when successive aliquots of the TPN mixture are introduced into a fixed volume of plasma. This static description does injustice to many aspects of the real system but would seem to provide the most satisfactory first order approximation as to how the ligand concentrations tend to be affected. There are several mitigating factors in favour of this approach: (i) pertinent experimental data is difficult to obtain, would vary considerably in accordance with the subject's nutritional status and could not be compared with the blood plasma of healthy individuals, (ii) powerful homeostatic controls are imposed on plasma component concentrations, and (iii) the metal-ligand interactions are generally very fast, whereas the clinical infusion rate is relatively slow. In all the calculations discussed here, the nutritive mixture most commonly used in the

TPN unit at the University Medical Centre of Poitiers has been adopted [C.Matuchansky, personal communication]. Its composition is depicted in Table 4.5.

The results of the computer simulations show that the percentage distributions in normal plasma (Table 4.3) are largely unaffected by the TPN infusion. This is because the input ligand concentrations tend to remain in a fixed ratio to one another. However, there is a rapid increase in the size of the low-molecular-weight fraction of zinc. The appropriate P.M.I. curve is shown as Figure 4.4. Also included in Figure 4.4 are the corresponding P.M.I. curves for the infusion of the three amino acids, cysteine, histidine and glycine, by themselves. It is clear that injection of the TPN fluid causes a greater relative mobilization of zinc from proteins than do any of the individual TPN components. Thus it would seem that the observed zinc excretion must be attributed to the additive action of two or more amino acids included in the fluid. Inspection of Figure 4.4 indicates these amino acids are probably cysteine and histidine.

It is noteworthy that in perfect agreement with the animal experiments of Yunice *et al.* [1871], the models predict cysteine will produce the greatest urinary zinc excretion, histidine will have a lesser effect and the influence of glycine will be almost negligible. The present results, however, argue against some interpretations made by the above-mentioned authors who concluded that the high excretion caused by cysteine was not due to an increase in filtered load. It seems highly unlikely

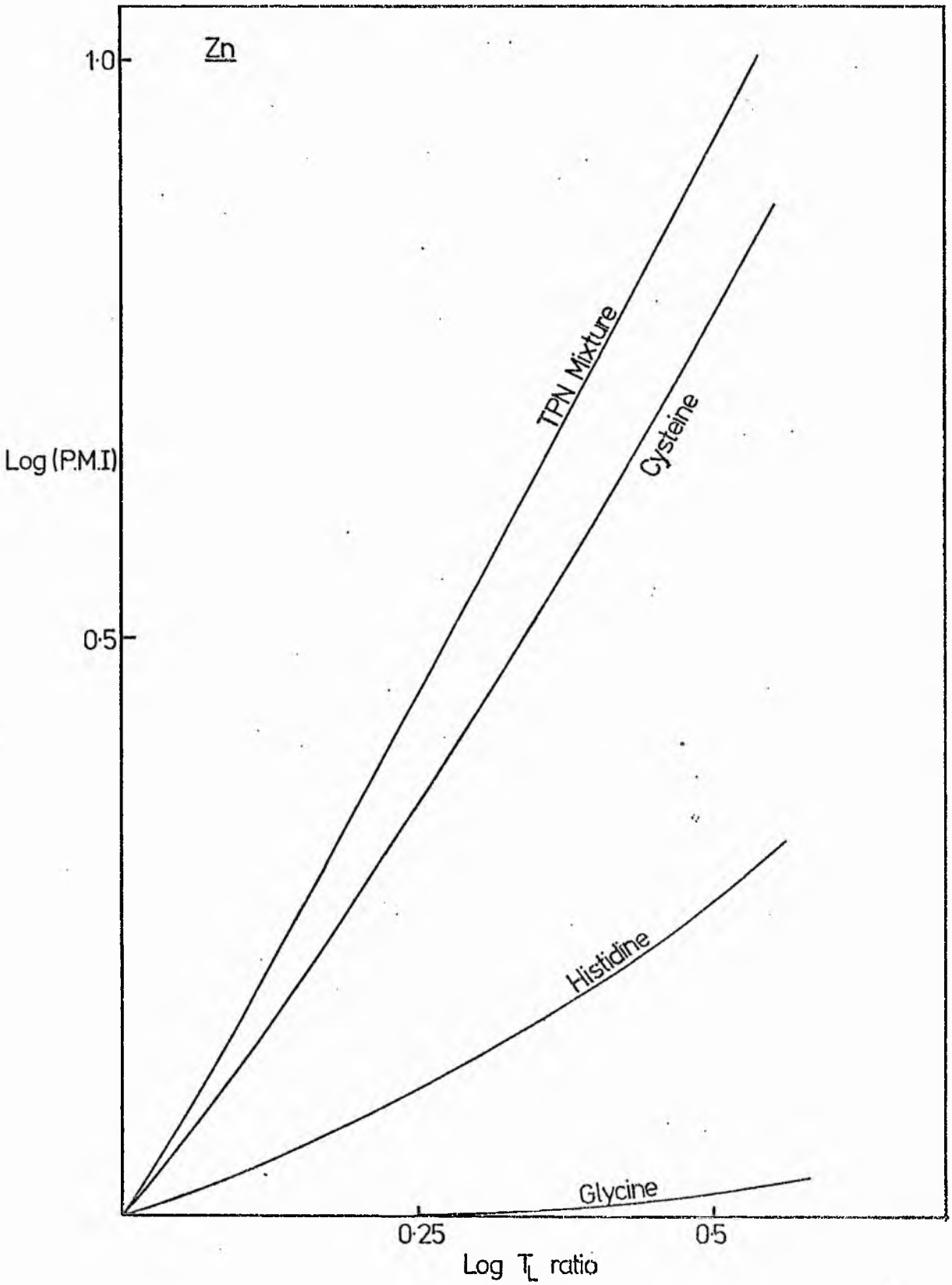
TABLE 4.5

Ligand composition of the nutritive mixture as used in the computer simulations

Ligand	Concentration (mmol dm^{-3}) (accurate to ca. 5%)
Alanate	20.66
Arginate	27.81
Aspartate	5.824
Citrullinate	3.319
Cysteinate	1.672
Glutaminate	6.586
Glycinate	23.23
Histidinate	6.245
Isoleucinate	7.534
Leucinate	20.53
Lysinate	18.32
Methionate	12.21
Ornithinate	3.428
Phenylalanate	14.66
Prolinate	13.47
Serinate	2.397
Threonate	8.135
Tryptophanate	2.467
Tyrosinate	0.2674
Valinate	20.68
Phosphate	5.170
Sulfate	0.9690
Lactate	15.50

FIGURE 4.4

(line width indicates error)



from the results that histidine actually binds more zinc in plasma than cysteine and this in turn suggests that their *in vitro* techniques were hampered either by the facile autoxidation of thiol compounds [1985] or by a disturbance of the metal-ligand equilibria during their ultrafiltration procedure. The incrimination of cysteine as the agent primarily responsible for excessive zinc losses indicates that nutritionists should look into the possibility of partially replacing this TPN component with more methionine. It has been established that the latter amino acid can be metabolically converted into cysteine [145,1890] so such a strategy may be able to minimise the effect of TPN infusions on zinc balance.

Following an idea suggested by Dr.G.Berthon, an attempt was made to obtain some quantitative idea as to how much of each trace element should routinely be added to TPN fluids. The object would be to replace the normal losses and compensate for the enhanced urinary excretion of the transition metal ions. The proposal was to simulate the equilibria in the exogenous fluid using the free metal ion concentrations that pertain in plasma. By mixing two solutions with identical values for each free metal ion concentration, a redistribution of the metal ions amongst their complex species would be avoided.

Thus, such an infusion would not disturb the normal metal protein binding in plasma. The increased low-molecular-weight complex formation would occur entirely at the expense of the metal ion supplement. On the other hand, if the free metal ion

concentrations in the fluid being administered are higher or lower than their corresponding concentrations in plasma, the metal-protein complexes will act to buffer each free metal ion concentration with the consequence that the prevailing balance would be shifted in favour of excretion or retention of the metal.

Tables 4.6 and 4.7 show the computed percentage distribution of the predominant metal species and the total metal concentrations for a TPN mixture having the composition shown in Tables 4.1 and 4.5. By far the most important transition metal complexes are formed by zinc with about 90% of this metal bound to cysteine, histidine and glycine binary and ternary complexes. Within the accuracy with which the free metal ion concentrations are known, the predicted amounts of total metal to be included in the infusate are in very close agreement with the operational values used at present [C.Matuchansky, personal communication]. Indeed, they lie within the experimental limits in every case. A daily calculated intake of 507 mg of calcium is to be compared with 500 mg actually administered. No doubt this eminently satisfactory agreement arises because the plasma free calcium ion concentrations are sufficiently high to have been measured directly by ion-sensitive electrodes [136,202]. Much greater attention has been paid to this alkali earth metal than to the transition elements so the above result provides the strongest indication both of the reliability of the model and of the simulation technique itself. With magnesium, the predicted dose lies between 200 and 400 $mg.day^{-1}$, whereas the real quantity

TABLE 4.6

Percentage distribution of Ca(II), Mg(II), Zn(II), Cu(II), and Mn(II) amongst their most predominant complexes in the nutritive mixture at $-\log[H^+] = 7.4$

% distributions are independent of the free metal ion concentrations to a precision of 1%.

Complex species	Percentage of the total metal
$[Ca(PO_4)]^-$	11
$[Ca(LysO)H_2]^{3+}$	10
$[Ca(LactO)]^+$	7
$[Ca(ProO)H^{2+}]$	5
$[Ca(ArgO)H]^{3+}$	5
$[Ca(GlyO)H]^{2+}$	3
$[Ca(PO_4)H]$	2
$[Mg(LysO)H_2]^{3+}$	16
$[Mg(ArgO)H]^{3+}$	11
$[Mg(GlyO)H]^{2+}$	5
$[Mg(LactO)]^+$	5
$[Mg(ProO)H]$	4
$[Mg(PO_4)H]$	4
$[Mg(LeuO)H]^{2+}$	3
$[Mg(AlaO)H]^{2+}$	2.
$[Zn(HisO)(GlyO)_2H]$	31*
$[Zn(CysO)(HisO)]^-$	18
$[Zn(HisO)_2(GlyO)H]$	12*

/contd

Table 4.6 (contd)

The ligands are represented by symbols as follows:

AlaO = alinate; ArgO = arginate; CisO = cystinate;
CysO = cysteinate; GluO = glutaminate; GlyO = glycinate;
HisO = histidinate; LactO = lactate; LeuO = leucinate;
LysO = lysinate; MetO = methionate; PheO = phenyla inate;
ProO = prolinate; ThrO = threonate; ValO = valinate.

The calculations are based on the free metal ion concentrations shown in Table 4.1 and on the total ligand concentrations for the nutritive mixture shown in Table 4.5.

*These values are probably too high. They are calculated from published formation constants [158] but following the usual approach towards complex species which appear to be significant, the zinc-histidinatoglycinato system is presently being investigated experimentally. Preliminary results suggest that lower formation constant values may be applicable [G.Berthon, personal communication].

TABLE 4.7

Total concentrations and predicted doses for Ca(II), Mg(II), Zn(II), Cu(II) and Mn(II) in the nutritive mixture at $-\log[H^+] = 7.4$

Metal	Total concentration (mol dm^{-3}) [both calculated to a precision of 1%]	Predicted dose (mg/day)
Ca(II)	4.903×10^{-3}	507
Mg(II)	4.070×10^{-3}	247
Zn(II)	1.914×10^{-4}	32
Cu(II)	7.857×10^{-6}	1.3
* Mn(II)	5.537×10^{-12}	10^{-6}

The calculations are based on the free metal ion concentrations shown in Table 4.1 and on the total ligand concentrations in the nutritive mixture shown in Table 4.5. The predicted dose is the calculated amount of each metal in 2.580 dm^3 of nutritive mixture, this being the usual volume administered daily.

* to be regarded as negligible.

administered is just outside this range at about 450 $mg.day^{-1}$. This large uncertainty in the case of magnesium arises because neither the free ion concentration in plasma nor the relevant formation constants are as well known as for calcium. The calculated daily amount of zinc lies between 25 and 50 mg. Although an average oral requirement of 20 mg is not strictly comparable, it suggests the model's predictions are of the correct order of magnitude. Furthermore, the calculated amount lies within the range employed by Kay and Tasman-Jones [1877]. For copper, the figure of 1.3 $mg.day^{-1}$ is a little higher than the 250-500 $\mu g.day^{-1}$ which is currently injected but is again within the limits suggested by Jacobson and Wester [1879]. As no signs of copper deficiency arise with supplementations at their present level, there is little incentive to recommend an increase. The very small predicted dose of manganese indicates that for the time being at least this element can be neglected.

One other application of this study which is worth mentioning briefly concerns the intravenous infusion of single component chelating agent solutions. Clinical indications for this kind of treatment arise when the drug cannot be given orally because of poor intestinal absorption [1849]. For example, this method is used to administer EDTA. It is of considerable practical value to redress the losses of essential transition elements which the chelation therapy induces. To illustrate this, consider a simulation of EDTA solutions comparable to those for the TPN fluid.

It indicates that zinc should be added to medical preparations of calcium disodium EDTA solutions in the ratio of 1 mole Zn^{2+} to 3 moles EDTA. This should be sufficient to prevent any effects arising from the zinc excretion often associated with this kind of therapy [883,979,1043,1048,1064,1085,1159,1160, 1210,1260,1522,1712].

In spite of the various above-mentioned successes, a number of the TPN-model's limitations must be borne in mind. Although the formation constants and total ligand concentrations are generally speaking the parameters most accurately defined, even these need continual reassessment and improvement. On the other hand, the greatest uncertainty in the model arises from an inadequate knowledge of the free metal ion concentrations in plasma. There are also difficulties associated with the redox equilibrium situation in biofluids. Most importantly, perhaps, is the assumption that perturbations of the metal-ligand equilibria in plasma will for the most part manifest themselves in a changed degree of metal protein binding. Of course, this will not be the only effect. There are also implications for the distribution of each metal into and out of the blood plasma compartment. Clearly, a large increase in the low-molecular-weight complex concentrations will not only provoke the enhanced urinary excretion of the metal ion but also tend to increase the amount of metal being deposited in tissues. However, if one considers that the infusions have metal ion supplements designed to maintain constant the binding ability of normal plasma towards each metal ion, it seems reasonable to assume that the body stores of the metal will not be dramatically affected.

4.2.4 Computer simulation of other biofluids

The computer models of metal-ligand equilibria described so far have had one very important characteristic in common. They all referred to solutions in which the composition of the low-molecular-weight fraction was fairly easily defined. The total ligand concentrations could be accurately determined and were invariant. More significantly, perhaps, the free metal ion concentrations tended to be well buffered. There are few biofluids which satisfy these criteria to the same extent as blood plasma. In many other cases, far less is known about the identity and abundance of the low-molecular-weight ligands although the situation is similar to blood plasma because metal-protein complexes occur at relatively high concentrations. Two examples are cerebrospinal fluid and cell cytoplasm. These are not of immediate concern, however, because as soon as the necessary data becomes available, very good models could be constructed using the simulation techniques already developed. On the other hand, there are some instances where it would be very valuable to simulate solutions even though very little can be said about the extent of metal ion interactions with biological binding sites. Intestinal fluid is a case in point. The search for ligands to promote the absorption of essential trace metals would be greatly facilitated if orally-administered solutions could be simulated satisfactorily.

In the past, attempts have been made to model metal-ligand equilibria in stomach and intestinal juices by considering only the reactions between the metal ion and the administered drug [312,316,861,1275,1603,1608]. This approximates best to administration on an empty stomach for it assumes that dietary and digestive components can be omitted since (i) the drug exists at relatively high concentrations and (ii) gastro-intestinal factors have a constant influence on a series of agents [1608].

Unfortunately, the second point is not always valid in the context of metal-ligand equilibria. Firstly, complex formation in the biofluid depends on the degree of endogenous competition for the metal ion. Secondly, the volume of liquid in which the drug is taken to have dissolved can never be more than arbitrary. Remembering that a diminished free metal ion concentration discriminates against polynuclear complexes and that dilution favours mono- over bis-species, it is clear that a unique complex distribution cannot be obtained under these circumstances.

One of the chief advantages with computer applications, however, is that it is often feasible to survey a range of possible solutions. Accordingly, this is proposed for those biofluids in which the concentrations of neither the ligands nor the metal ion can be easily resolved.

There are two possible alternatives which might form a basis for computer simulation of intestinal fluid. On the one hand, the interaction between the metal ion and the biological binding sites may completely remove a portion of the metal ion

available to the drug. Conversely, the effect may be more akin to the binding by protein molecules which has previously been described as a means of establishing a metal ion reservoir. The first situation can be simulated by considering the total metal ion concentration, whereas, in the second case, the free metal ion concentration is a more appropriate parameter.

Thus, by scanning both the free and the total concentrations of a metal ion in the presence of a given concentration of drug, the entire profile of complexes which can be formed will be covered. More importantly, when two drugs of the same concentration are compared, these scans of metal ion concentration represent the two extreme possibilities which ought to be considered. Of course, this does not discharge the need to consider the effects of each drug at different levels: the metal ion scans will need to be repeated at a variety of ligand concentrations.

In this way, two representations of the complex distribution for every system can be obtained. The amount of each complex which is formed is expressed as a function of the total ligand and either the total or the free metal ion concentration. It is then possible to compare agents according to any well defined criterion. As biological interactions will be considerably greater for transition metal ions than for ligands, it is reasonable to make such comparisons on the assumption that the different agents exist in the biofluid at the same total concentration, but even this is not strictly necessary.

To illustrate how this concept may be utilized, consider the intestinal absorption of a metal ion and how this may be promoted by a series of chelating agents. Ligands which form the highest concentration of neutral complexes in the intestinal fluid are expected to enhance passive diffusion of the metal ion across the epithelium most successfully. Hence, each agent is ranked according to this criterion using both free and total metal ion scans at an appropriate free hydrogen ion concentration. The order obtained from each scan will rarely conflict but when they do so, the effect is merely to introduce some ambiguity into the model's predictions. The same can be said for the comparisons made at various total ligand concentrations. The outcome is a decisive classification of the agents into groups of one or more according to their predicted ability of promoting the absorption of the metal ion. This can be unequivocably checked against animal screening experiments. The procedure is demonstrated in Example 4.1.

4.2.5 Computer simulation and the role of copper in rheumatoid arthritis

"It has been said that everything is known about rheumatoid arthritis except its cause, natural history and treatment. It is one of the perversities of the human condition that the rarer the disease, the more clearly it seems to be understood....."

R.C.Williams
(Ref.1987)

EXAMPLE 4.1 SIMULATION OF INTESTINAL FLUID

Classification of chelating agents according to ability to promote intestinal absorption of transition metal ions

Kratzer and co-workers [888,1232] have studied the effect of various chelating agents on the absorption of zinc ions from soya bean protein as reflected in poultry growth rates. Their results for those ligands which have been included in the computer database of formation constants (Appendix A2) may be summarised as follows:

Ligand	Percentage weight gain
Hydroxyethylethylenediaminetetraacetate (HEDTA)	120
Ethylenediaminetetraacetate (EDTA)	100
Nitrilotriacetate (NTA)	68
Ethylenediaminediacetate (EDDA)	64
Diethylenetriaminepentaacetate (DTPA)	26
Cyclohexanediaminetetraacetate (CDTA)	8
Triethylenetetramine (TETA)	8
Prolinate (PRO)	8
Glutamate (GLU)	0

Makar and Williams [2032] have recently considered Kratzer's experiments using computer simulation models and the following analysis can be considered as an extension of their work.

Both total and free zinc concentrations were scanned for each ligand as described in the text to check that the relative complexing ability of the chelating agents was independent of the values chosen. This was found to be the case. Representative results using arbitrarily chosen zinc concentrations are shown in the following tables. Note that these represent two extreme cases: the first is most applicable when there is no competition from endogenous binding sites and the second refers to situations in which the free metal ion concentration is perfectly buffered by proteins.

TOTAL ZINC CONC. = 10^{-8} mol dm^{-3}

Ligand	Log of species concentration as a function of charge					
	≤ -3	-2	-1	0	+1	+2
DTPA	- 8.0	- 9.0	-11.1			
EDTA	-12.5	- 8.0	-11.1			
HEDTA			- 8.0	-12.5		
NTA	- 9.4	-11.6	- 8.0			
CDTA		- 8.0	-12.7			
EDDA		-15.2	-10.8	- 8.0		
PRO			-13.5	- 9.9	- 8.8	
GLU	-13.1	- 9.5		- 8.3		
TETA						- 8.0

/contd

FREE ZINC CONC. = 10^{-15} mol dm^{-3}

Ligand	Log of species concentration as a function of charge ⩽-3	-2	-1	0	+1	+2
DTPA	- 4.9	- 5.9	- 8.2			
EDTA	- 9.2	- 4.7	- 7.9			
HEDTA			- 5.6	-10.1		
NTA	-10.9	-13.0	- 9.4			
CDTA		- 3.9	- 8.6			
EDDA		-16.2	-11.8	- 8.9		
PRO			-20.0	-16.4	-15.3	
GLU		-16.3		-16.1		
TETA						-11.59

It is evident from Kratzer's results that the zinc is able to be transported across the epithelium as complexes which are not necessarily neutral. It therefore seems reasonable to assume that it is the concentration of species with low charge density (+1, 0, -1) that will determine how much zinc is assimilated. Accordingly, the computer simulation results can be condensed as follows.

Ligand	Log of the concentration of species with low charge density (+1, 0, -1)	
	T_{Zn} = 10^{-8} mol dm^{-3}	$[Zn^{2+}]$ = 10^{-15} mol dm^{-3}
DTPA	-11.1	- 8.2
EDTA	-11.1	- 7.9
HEDTA	- 8.0	- 5.6
NTA	- 8.0	- 9.4
CDTA	-12.7	- 8.6
EDDA	- 8.0	- 8.9
PRO	- 8.8	-15.3
GLU	- 8.3	-16.1
TETA	-	-

The table shows that HEDTA will, under all circumstances, carry the metal as a complex of low charge density to a greater extent than any of the other agents under consideration. In this respect, moreover, the ligands EDTA, DTPA and CDTA are found in the same relative order whichever model is used. Hence, it can be predicted that to promote zinc assimilation EDTA will be better than DTPA which in turn will be superior to CDTA. Finally, the models show that TETA must always be amongst the agents with least effect because it is unable to form complexes with a charge less than +2. In all these instances, the model's predictions are in accord with Kratzer's experimental data.

The term rheumatism is generally used to refer to painful disorders of the joints and muscles which cannot be directly related to an identifiable infection or injury. There is actually a wide range of connective tissue diseases which are rheumatoid in nature [1987-1989]. The most common of these is rheumatoid arthritis. It afflicts over 5% of the population of the western world [1988] and is particularly prevalent amongst the elderly. It usually manifests itself as small nodules of inflamed fibrous tissues around the knuckles and wrists, eventually causing irreversible functional damage to the joints.

The origins of rheumatoid arthritis are unknown but many suggestions centre upon a breakdown of the patient's autoimmune system [1987,1988]. It seems that, for one reason or another, an anti-inflammatory response to physical, emotional or hormonal stress is not properly regulated by the usual feedback mechanisms and the irritation thus continues to stimulate itself. It is clear that the disease is a complicated expression of many interrelated cellular and molecular processes. So, in common with many pathologies which stubbornly resist modern medical efforts, it is difficult to remedy because there is no unique and identifiable biochemical lesion [1987]. However, agents which suppress the inflammation interrupt the self-perpetuating process and can thus be used to control the disease if not to cure it. This explains why the pain can be alleviated by a multitude of immunosuppressive agents as well as salicylates, certain acid drugs and, more recently, metals.

4.2.5.1 Copper and inflammation

The fact that copper occurs in plant and animal tissues was reported in 1816 [1990] but its essentiality was not established until 1928 when copper-deficient rats were shown to develop anaemia [1991]. Dietary deficiencies were subsequently found in several larger animals [1992]. There followed a host of reports about disorders which arose from insufficient dietary copper, ranging from bone deformation to cardiac malfunction [403,1993]. Fortunately, the element is so widespread in nature that the daily intake of most mammals more than satisfies their requirements. A general, nutritional copper deficiency in man is thus exceedingly rare but there is now much evidence that localised copper imbalance is related to rheumatoid arthritic conditions.

For many years this evidence remained somewhat circumstantial. Elevated concentrations of total serum copper are very characteristic of the disease [470,491,494,496,1511,1970,1971,1996, 2001,2003] but the cause of this, an increase in ceruloplasmin levels [491,1972,2005] is observed in many inflammatory conditions and may only be secondary to this kind of pathology [1969,1973, 1995,1996,2004,2006]. Likewise, many folk-lore remedies for rheumatoid arthritis (most notably copper bangles) have an obvious link with trace element but it is difficult to prove that they confer any real benefit. The amount of copper involved is usually very small so most assessments become dominated by psychological factors. The attitude of patients, for example, is often substantially determined by their first experience of a treatment

and thus coincidental remissions, due to characteristic oscillations in the severity of the disease, are prone to make very strong impressions. Indeed, it can be argued that any new approach prompted by an acute bout of suffering is more than likely to appear efficacious.

However, there is now overwhelming evidence that a direct connection between copper and rheumatoid arthritis exists. The essence of this is that (a) low-molecular-weight copper concentrations in plasma and synovial fluids are increased as part of the body's natural response to the disease and (b) when such increases are induced by copper administration they are observed to have a most potent antiinflammatory effect.

The antiinflammatory activity of a multitude of copper compounds has been demonstrated by several investigators using a wide variety of animal screens [754,1005,1273,1603,1740,2007]. Although there is no completely satisfactory animal model of rheumatoid arthritis [2008], these results strongly suggest that it is in the control of inflammation that copper primarily manifests itself against the disease.

There are sharp increases in both the ceruloplasmin and non-ceruloplasmin concentrations in response to infections, irritants and inflammation [403,1996,2002,2009,2010]. Lorber *et al* were the first to report statistically significant elevations of total serum copper concentrations in rheumatoid arthritis [491]. In normal individuals ceruloplasmin accounts for most of the total [490,580,999,1466,1510] but in those suffering from rheumatoid arthritis there is a large increase in the non-ceruloplasmin fraction [491]. Certain findings that contradicted

the above generalisation [489] have been attributed to the methodologies used [490] but as Sorenson has pointed out [1740] they may also reflect differences in the activity of the disease in each of the sampled populations. It seems likely that the increased non-ceruloplasmin and hence increased low-molecular-weight copper concentrations may arise from an accelerated turnover of ceruloplasmin during arthritis [2005].

The first report that a low-molecular-weight copper complex was effective against rheumatoid arthritis appeared as early as 1941 [2011]. Sorenson and Hangarter have reviewed the treatment of some 1500 patients since then with a variety of copper compounds [1611]. These preparations all bring about marked reductions in inflammation and Permalon particularly shows very few toxic reactions. Indeed, they exhibit an anti-ulcer activity [744,1273] which is significant because gastrointestinal irritation commonly necessitates treatment by many anti-arthritic drugs to be abandoned [1740]. This is in line with the role of copper in generally preventing gastro-intestinal damage by acidic anti-inflammatory agents [1337,2012].

Sorenson has also reviewed a great deal of additional evidence concerning copper complexes as anti-arthritic drugs [1740] so only two pertinent examples that have arisen recently will be mentioned here. First, speculation about the therapeutic efficacy of copper bracelets has been placed on a more scientific footing: the dissolution of metallic copper in human sweat has been quantified [1127], its dermal penetration studied [1470] and its positive beneficial effects against

rheumatoid arthritis demonstrated in a clinical trial [1127]. Second, the need to maintain a minimum level of copper in tissues for the control of inflammation has been established by Milanino *et al* who showed that copper-deficient rats were significantly more susceptible to carrageenan-induced oedema than control animals [2013].

As stated previously, the mechanism by which copper acts as an anti-inflammatory and anti-arthritic agent is still unknown but many plausible hypotheses have been put forward. Most conventionally, the metal may affect the balance between the prostaglandins PGE_2 and PGF_2 which regulate the autoimmune response [779,956,2014]. The anti-inflammatory activity of PGF_2 is stimulated by the presence of Cu(II) [779] possibly because the metal is required for its biosynthesis. Alternatively, Cu(II) may reduce the availability of glutathione, one of the PGE_2 precursors [779] or inhibit fatty acid dioxygenase (which initiates the conversion of unsaturated acids to prostaglandins) [950,1042].

Other possible roles for copper involve its metallo-enzymes. Lysyl oxidase, needed for collagen and elastin synthesis [480,785,984,1254], has attracted much attention because of the potential pathological significance of defective connective tissue synthesis in rheumatoid arthritis [1740]. Similarly, diminished concentrations of the copper enzyme superoxide dismutase may permit increased synovial tissue irritation by hydroxyl and/or superoxide radicals [965,1001,1002,1958]. Copper may also participate in the stabilization of membranes, especially the lysosymal membrane, by maintaining disulphide linkages in their oxidized form. Although it is unlikely that Cu(II) ions would

ever be present in sufficient concentration to achieve this by themselves, the biological mechanism may well invoke oxidation by some copper-containing species.

It has been suggested that the effect of copper may be due to tissue irritation at the site of injection [1827,2015]. Indirect suppression of an experimentally induced kaolin or carrageenan-oedema would be expected in the face of a natural anti-inflammatory response. This may involve corticosteroids or even copper itself [1740]. However, the effect is neither sufficiently large nor sufficiently related to copper's anti-inflammatory activity to account totally for it [754,1603]. Nevertheless, it does raise the important point that unless ligands are both powerful and specific binders of the metal, in most biofluids dissociation of the complex is likely to occur. In extreme circumstances, copper complexes will thus exhibit the well known toxic effects of copper salts, albeit to a lesser extent.

Too little attention has been paid in the literature to the labile nature of many copper complex systems in aqueous solution. A surprisingly large number of solids have accordingly been prepared (and structurally analysed!) before administration when a simple mixture of the ligand and copper salt would equally have sufficed. Conversely, experiments designed to demonstrate the effectiveness of pre-synthesised complexes over their components rarely, if ever, take sufficient account of either the effect of dissolution or of the species which are subsequently formed.

An important example of this concerns the complexes of copper and salicylate. Many studies, carried out on both animals and humans, have concluded that a copper salicylate species is the active anti-rheumatoid agent [754,1049,1611,2016, 2017]. This seems unlikely in view of the general anti-inflammatory properties of copper exhibited with a wide variety of ligands. It also neglects the fact, revealed by computer simulations of the relevant metal-ligand equilibria, that such complexes are thermodynamically unstable in plasma. If they enter the biofluid as solids, the tendency will be for them to dissolve and, once in solution, they will rapidly dissociate. Arena *et al* have thus concluded that the observed synergistic effects shown by copper salicylate over its components arise because the complex formed in intestinal fluid is neutral and hence facilitates the absorption of the metal ion [1607]. It is also conceivable that a slow dissolution of the solid might itself be beneficial if it served to maintain a low but constant concentration of the neutral species in solution.

These computer-based predictions about the fate of most copper complexes introduced into plasma are strongly in accord with the antiinflammatory screening data available for a wide range of copper-ligand complexes and mixtures [754,1603]. When the preparations are administered subcutaneously (i.e. in such a way that they can pass into plasma without having to traverse a membrane) a striking correlation emerges between the total amount of copper injected and the observed reduction in inflammation [1603]. This implies that the nature of the complex formed by the variety of ligands concerned, does not influence the result.

Rather, it is the increased availability of copper ions *per se* which affords protection against the inflammation. Whatever the mechanism through which copper acts, it is able to aquire the metal ion from the labile equilibrium system in plasma once the complex has dissociated and the metal ion been distributed proportionally amongst serum albumin and the naturally-occurring low-molecular-weight ligands (see Section 4.2.1.).

In striking contrast to the results obtained when the copper complex solutions are administered subcutaneously, oral doses proved almost totally ineffective [1603]. From this it was concluded that the complexes in question were not sufficiently robust to be absorbed intact through the stomach or intestinal membranes. Natural homeostatic mechanisms [822,827,1839] evidently compete with the labile metal species to prevent excessive assimilation of the trace element. This underlines the idea that it is not a general copper deficiency that needs to be treated but rather a localised imbalance which can be corrected by an overall supplementation. The principles by which these homeostatic processes may be bypassed in order to introduce therapeutic amounts of copper into plasma without resorting to direct injection are discussed in Section 4.2.5.3.

4.2.5.2 Copper, rheumatoid arthritis and penicillamine

The simulation techniques pertinent to rheumatoid arthritis that were discussed in Section 4.2.4 are still too new to have demonstrated their full potential. However, in one major respect, computer models have already proved to be a valuable tool for

unravelling the relationships between the metal ion and ligands involved in the treatment of this disease. The instance concerns the drug penicillamine (β-mercaptovaline).

It was in the treatment of Kinnear Wilson's disease that penicillamine was first clinically applied. This affliction arises from a defect in the mechanism through which copper is excreted in the bile [1013]. Untreated, it leads to an increasing copper overload that is ultimately always fatal. In 1954 Walshe discovered that penicillamine produces an extraordinary increase in urinary copper excretion and so offered hope to many who would otherwise have been condemned to progressive degeneration of the nervous system and finally to the failure of organs such as the liver [341,1013]. A host of investigations concerned with the interaction between penicillamine and copper metabolism have followed [884,944,1021,1047,1050,1112,1119,1191, 1285,1718,1720].

Since then, penicillamine has also been introduced for a wide variety of other medical purposes [492,1010,1625]. It is the chelating agent of choice for many heavy metal poisons [1012,1014,1019,1039,1048,1058,1071,1095,1096,1097,1117,1118, 1322,1343,1721,1755,1881,1887]. Most recently, it has become established as one of the most effective means of combating rheumatoid arthritis [1105,1236,1259,1538,1576,1627].

The mode of action of penicillamine in the treatment of rheumatoid arthritis is a complicated one [2019]. The drug prevents connective tissue proliferation and the accummulation of rheumatoid components [1502,1505,1961,1962,2019] but it is

not known with certainty how it does so. Jaffe showed that there is a latent period before a course of treatment comes into full effect and that its influence persists long after discontinuation [1105,1964,1965,1966]. The pharmacokinetics of the compound are not responsible for this [917,1061] so that hypothetical mechanisms based solely on a simple depolymerisation reaction [1962,1966,1967] or on the inhibition of a single biosynthesis [985,988,1505] are, at least, incomplete.

One aspect of the anti-rheumatoid activity of penicillamine may be exerted through its effect on copper metabolism. Such a connection was originally explored by Gerber [493,1109,1612] and has since gathered much support. At first sight, the excretion of copper caused by the drug may appear to argue against this for inflammation is controlled by copper administration. However, the paradox disappears if one considers penicillamine to be, primarily, a copper mobilizing agent. It thus results in an enhanced urinary excretion of the trace element but may also temporarily increase the concentration of copper in the tissues.

This leads to the question of how penicillamine mobilises copper. The compound has become something of a panacea for treating heavy metal toxicity so it is easy to conclude that powerful chelation lies at the heart of its effect. In other words, one assumes that it acts upon the labile copper equilibria in plasma in the same way as it does for other transition metal ions like cadmium, mercury and nickel [1003,1016,1285,1718].

The information which can be obtained from computer simulations of penicillamine in blood plasma suggests otherwise. There is a clear distinction between the interaction of this ligand with copper and with other metal ions. As may be seen from the P.M.I. curves shown in Figure 4.3 (p.109) Trien (triethylene-tetramine) is the most powerful of the copper binding agents studied. Penicillamine exhibits similarly large P.M.I. values at relatively low drug concentrations with zinc and lead. This suggests that the drugs are capable of transferring these metal ions from serum albumin into the low-molecular-weight fraction. The charged complex formed by Trien (CuL^{2+}) is clearly amenable to renal excretion, as is indeed observed [944].

The manner by which penicillamine produces its copious cupruresis is not so straightforward. As the vast majority of exchangeable copper ions in plasma are bound to serum albumin in their divalent state, the drug cannot interact with them without being oxidized [369]. On the other hand, when the oxidised form of the ligand is compared with Trien, it is evident that it is a relatively weak chelator of copper(II) under the conditions pertaining in blood plasma. This computer-based prediction is substantiated by the fact that penicillamine disulphide does not itself increase copper excretion [1322].

The traditional biomechanism claimed to cause the copper excretion is that of 'reductive chelation' [737]. At best this is an oversimplification because a second molecule of reduced penicillamine would be required to bind the reduced copper(I) ion after it had been liberated from the protein.

In fact, the labile nature of the equilibrium means that special mechanisms for the metal's release from serum albumin [269,737] are superfluous. Moreover, the feasibility of copper(I)-penicillaminate complexes existing in blood plasma at concentrations sufficient to give this drug its unique bioinorganic properties seems doubtful [1048,1112,1343]. There is a large surplus of ligands in plasma which prefer to bind copper in its higher oxidation state and furthermore, the penicillamine would have to compete for copper(I) ions against high naturally-occurring levels of cysteine [666].

Such a possibility has not yet been fully assessed by computer model calculations for it would require a complete thermodynamic appraisal of the oxidised and reduced forms of cysteine as well as penicillamine reacting with both oxidation states of copper. Some of the necessary data has recently been determined by Österberg *et al* [2020] but further experimental work and more calculations are still required. For the moment, however, the verdict must be that the role of copper(I) complex formation is no more than borderline: it is probably not crucial to the mechanism by which penicillamine promotes copper excretion.

Mention should be made of the red-violet, 'mixed' copper complex $[Cu(II)_6Cu(I)_8Pen_{12}Cl]^{5-}$, the chemistry of which has been widely studied under the assumption that it is biologically relevant. This was implied by early experiments in which this polymeric species was identified in the plasma and urine of animals [1128]. As the experiments commenced with the administration

of the complex, in fact they provided no evidence that it was formed *in vivo*. Computer model studies were the first to suggest that this could not happen in blood plasma because of the extremely low metal ion concentrations occurring in the biofluid. It was shown experimentally that the same was true of the urine [1474]. A comprehensive appraisal by Laurie and Prime [2021] has now confirmed these conclusions. Thus, if the complex is ever formed *in vivo* it must be within cells where the redox potential and absence of serum albumin may permit sufficiently high levels of copper(I) and copper(II) ions but even this possibility should be regarded with considerable reservation. On the other hand, the slow release of both copper and penicillamine from this or similar complexes that will occur after their administration into plasma may be a means of producing a two-edged attack on rheumatoid arthritis.

The question thus remains: what is the *modus operandi* of penicillamine on copper metabolism and where does it act? Recent reports increasingly suggest that the source of the copper is the liver [1659,2022]. As opposed to Trien which depletes plasma copper levels until a certain limit is reached, penicillamine causes an excretion of copper proportional to the drug's dosage and there is a simultaneous reduction in liver copper concentrations [2022]. These observations are consistent with the idea that Trien competes for the limited amount of the metal ion bound to serum albumin whereas penicillamine taps a much larger supply.

As penicillamine and triethylenetetramine derive copper from different body compartments [944,1285], it seemed likely that simultaneous administration of the two agents would produce a synergistic cupruresis. This was tested in a recent investigation by Planas-Bohne but was not confirmed [2022]. It appears that there is a definite limit above which higher doses of either triethylenetetramine or triethylenetetramine and penicillamine do not cause greater copper loss. This is understandable if the rate of excretion is governed by the capacity of the labile equilibrium system in plasma. Perhaps the synergistic effect will be observed at drug concentrations which do not exceed the above-mentioned limit. If so, the required doses of penicillamine used to treat Wilson's disease may be reduced and thereby help to minimise adverse reactions to it.

Another pertinent interaction of penicillamine has just been established by Pickup *et al* [2025]. They found that unlike patients on other anti-arthritic therapies, those taking this compound had significantly raised histidine concentrations in their blood plasma. Their observation is of particular interest because Gerber has demonstrated that concentrations of free histidine are characteristically depressed in rheumatoid arthritis [2023,2025] and that this permits an aggregation in synovial fluid of degraded gamma globulin [1109] being both inflammatory and antigenic [1963,2026]. The sulphydryl-dependent denaturation of gamma globulin is inhibited by mixtures of cystine, histidine and copper(II) as well as by penicillamine disulphide and copper(II) [1109,1612]. So it seems that complexes of the metal

ion lie at the crux of at least two possible mechanisms by which penicillamine may suppress the immune response. Computer models have played a striking role in elucidating these relationships: they not only identified copper(II) cystinate histidinate as the most appropriate ternary species for experimetal consideration, but also showed that, of all the naturally occurring amino acids, changes in plasma histidine concentrations will do the most to disturb the copper ion equilibrium with the tissues (see Section 4.2.1.).

The unique action of penicillamine on copper metabolism is likely to depend on a property that distinguishes it from other chelating agents particularly the very similar, naturally-occurring mercapto-amino acid, cysteine. There are at least three possibilities to bear in mind. First, penicillamine may have an effect when cysteine does not because it is not homeostatically regulated. Second, it may disrupt copper-metalloprotein synthesis by being incorporated erroneously instead of cysteine. Third, it may specifically reduce and therefore extract copper from some inert metalloprotein.

The possibility that penicillamine might be removing copper from plasma ceruloplasmin has been investigated [1602]. Copper liberated from this metalloprotein could cause a very significant increase in the amount of the metal ion bound to serum albumin and to low-molecular-weight ligands. Serum and ceruloplasmin solutions were treated with a variety of chelating agents including penicillamine and then separated into different fractions by ultrafiltration. Although the chelating agents did remove copper

from the metalloprotein, a comparison of their relative abilities to do so very strongly suggests that copper mobilization by penicillamine *in vivo* does not occur by such a mechanism.

One potential target for penicillamine is obvious: metallothionein in the liver is normally rich in both zinc and copper [990,1635,1636,1637,1785,1790,1791,1833]. It is tempting to speculate that an ability to remove transition metal ions from metallothionein may be partly responsible for the striking success of this drug in the treatment of heavy metal poisoning.

It is clear that an improved understanding of penicillamine biochemistry would be valuable in many medical contexts. This section has focussed only on how the drug interacts with copper metabolism yet, in spite of all the research devoted to this question, there remains plenty of scope for further investigation.

4.2.5.3 Designing copper complexing agents for the control of inflammation

The *ab initio* design of therapeutic agents usually depends on a knowledge of the difference between health and disease at a molecular level. As the causes of rheumatoid arthritis are not yet well understood, it may therefore seem that a complete cure is not likely to be soon forthcoming. However, the observed effect of copper on inflammation provides a basis which can be utilized in the meantime. Whatever the mechanisms by which the metal achieves its observed biological effects, the aim clearly should be to increase the supply of copper at the sites of injury.

The rationale for designing rheumatoid arthritis agents which reduce inflammation in this way depends on two fundamental assumptions:-

(1) the therapeutic effect of copper administration arises from an increase in the total labile copper concentration in body compartments such as the synovial fluid, and

(2) this increase is fostered by the formation of complexes in plasma that can diffuse into the synovial fluid, i.e. through the separating membrane.

From the computer simulation results summarised in Section 4.2.1 it is evident that the supply of copper to the tissues will be enhanced simply by increasing the labile copper concentration in plasma itself. Unfortunately, the use of injections as the most straightforward way of accomplishing this objective is too hazardous to be a practicable therapeutical solution.

Humans can tolerate relatively large amounts of orally-ingested copper but very much less when the metal is intravenously administered. For example, copper sulphate is widely used as an emetic in doses as high as 1 g [2027] whereas a limit for intravenous injection of less than 1 mg dm^{-3} of blood plasma has been suggested [2028]. This is because under optimum circumstances less than one third of dietary copper is assimilated [610,1466,2029,2030] and this fraction is rapidly transported by serum albumin to the liver for ceruloplasmin synthesis. On the other hand, copper injections are likely to exceed the binding capacity of albumin (albeit locally) and so the metal ions attach themselves to non-specific protein sites. This leads to a much wider bodily distribution of the administered

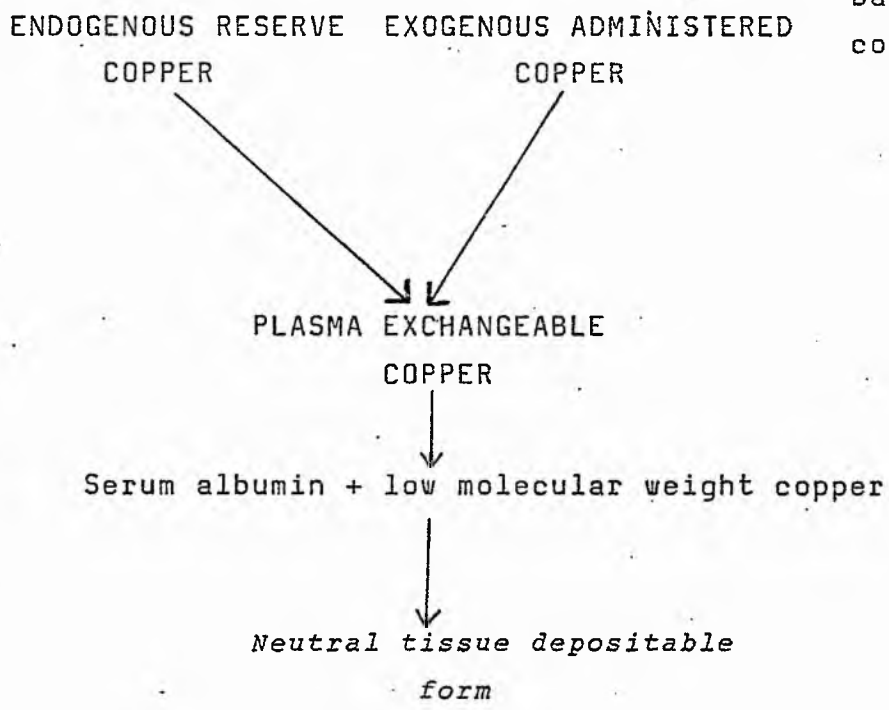
metal and to haemolysis of red blood corpuscles.

Although it is possible to alleviate these undesirable effects by giving the injections slowly or subcutaneously and by using chelating agents to help attain equilibrium binding, such a means of administering copper remains too inconvenient and too problematical to be widely adopted for rheumatoid arthritis treatment. Other ways of delivering the metal to inflamed tissues are therefore required. Figure 4.5 shows two general routes by which this may be accomplished. One involves copper supplementation by oral or topical administration. The other is based on liberation of endogenous reserves of the metal stored in the liver or elsewhere.

For short term therapy, aimed at correcting a localised copper imbalance, endogenous rather than exogenous sources seem to offer the simplest solution. There are three ways of achieving this objective:- (1) by equilibrium competition for labile protein bound copper, (2) by decreasing the affinity of serum albumin for copper by allosteric effects, and (3) by extracting copper from inert metalloproteins. It was suggested in Section 4.2.5.2 that penicillamine may operate by the latter mechanism. Fiabane and Williams [1604] have shown that many anti-rheumatoid drugs, such as Indomethacin, Naproxen, Ketoprofen and Fenoproten are capable of freeing copper bound to albumin by distorting the protein molecule and that there are certain parallels between their *in vitro* results and the efficacy of these agents in animal screens. It is not yet known whether this is part of the drug's action in man.

FIGURE 4.5

Scheme of routes for increasing labile copper in tissues



Enlarging the low-molecular-weight copper fraction in plasma by taking the metal from endogenous sources will cause a metabolic redistribution of copper at the ultimate expense of its storage metalloproteins. In part, this is because the strategy will inherently increase copper excretion, most probably in the faeces. Sooner or later, therefore, all such approaches will require copper supplementation.

The evidence reviewed in Section 4.2.5.1 shows that orally administered copper can be beneficial even when little thought has been given to how well the metal ion is absorbed. Applying the principles outlined in Section 4.2.4, one should thus be able to substantially enhance the effect of copper supplementation by a careful choice of ligands to maximise the amount assimilated. Factors such as the lipophilicity of the ligand and the stability and charge of the predominant complex formed in intestinal fluid clearly need to be considered. With lipophilicity, a balance must be struck between the ease with which the species penetrate biological membranes and their consequently increased toxicity. Similarly, the complex formed in intestinal fluid must be sufficiently robust or inert to withstand the competition by the metal binding species in mucosal cells which homeostatically regulate copper absorption but, on the other hand, the complex must not be so powerful that it doesn't dissociate after it has entered plasma.

To bypass some of these difficulties, attention has been drawn to the possibility of copper supplementation by dermal

application. The effect of copper bangles arises from the dissolution of the metal by ligands present in perspiration [1127,2031] but as the complexes need to penetrate the epidermis before they can be transported into plasma via the lymph, similar considerations to those discussed for intestinal absorption apply.

On the other hand, dermal assimilation has the advantage that there is no homeostatic control mechanism to be overcome. Thus, suitable complexes will not need to be so specific for copper(II) or so powerful. Gastrointestinal irritation by the metal ion is also avoided. For these reasons, topical applications of copper complexes for the treatment of rheumatoid arthritis appear most promising.

Although copper complexes have been used to treat rheumatoid arthritis for nearly forty years, it is only recently that they have begun to attract widespread interest. Pharmaceutical preparations involving copper are now available but have so far not been commonly employed in rheumatoid arthritis clinics. This is partly because the medical profession is somewhat reluctant to follow a line so long advocated by folk-lore but also because of the difficulty in demonstrating the therapeutic effects of copper ions without directly injecting them in solution. Hopefully this will soon be resolved by the improvement of topical and oral agents. Such progress will only be made possible by a better understanding of how to manipulate the *in vivo* concentrations of transition metal ions generally. It is in this respect that computer simulations will make a major contribution.

CHAPTER FIVE

CONCLUSION

5.1 Computer applications, past, present and future

Perhaps the most striking feature of the computer applications described in this thesis is their wide variety. Programs have been developed both to assist in the determination of metal-ligand formation constants and to model a number of systems with bioinorganic implications. Compared to the many problems which have been addressed, however, few are resolved. This is particularly true of the computer simulations because of the fairly broad objectives which were initially set. On the other hand, a start has been made which, in many respects, can be considered most encouraging.

Some may feel that the non-experimental nature of the models means that, at best, they can only provide incidental detail about the behaviour of transition metal ions *in vivo*. Such a view underestimates the role of models in science. Their purpose is to make predictions from hypotheses. By comparing the results of experiments against their simulated counterparts, it is possible to distinguish between good and bad ideas. In this way, complementary hypotheses that withstand the test of time can ultimately be fashioned into grander theories to account for a variety of natural phenomena.

Then, there are other, albeit less fundamental, attributes of models that ought not be forgotten. For example, they provide a background against which new experiments can be devised and/or evaluated. Unexpected results usually arise because the theory is incomplete or because of an experimental artefact. In the first case, systematic differences between the calculated and

observed quantities may suggest ways to improve the theory and in the second case, the situation is often made clearer because all the important relationships in the system already need to have been carefully formulated.

The emphasis which computer simulations of metal-ligand interactions in biofluids have placed on the role of equilibria and kinetics in the metabolism of trace elements will probably prove to be their greatest accomplishment. Overly fascinated by the spectral characteristics of transition metals, chemists have tended to forget that evolution is swayed more by their coordination and redoxing properties [1975]. The models have provided considerable insight regarding the chemical context in which all bioinorganic processes must occur.

A majority of the models' most specific achievements have been of a negative nature. A common example concerns metal complex species which are postulated to exist in plasma. Provided the formation constants are known, the relative ability of the complex to exist in the biofluid at equilibrium can often be reliably calculated. It may then be possible to state that such a species cannot form spontaneously from its components under the particular conditions. On the other hand, it can never be claimed so emphatically that a particular complex does predominate or even exist for, unlike the previous conclusion which is relative to other species in the solution, to be certain of the positive predictions requires that all the important interactions have been taken into account. Only when the performance of the models has been substantiated over a period of many years,

can confidence in their more positive predictions be considered justified.

As to the future, it would seem that the present equilibrium simulations are only a beginning. A thorough understanding of normal transition metal metabolism, how it is modified in disease and how it may be manipulated to restore good health is no longer just a dream. The stage is now set. How soon the goal is realised depends largely on how soon bioinorganic scientists learn to apply current knowledge to medical problems *at a molecular level*.

One contribution to this end would be the further development of models for the simulation of transition metal ion interactions in biological systems. Specifically, these should include a better description of (i) metal binding by proteins, (ii) kinetic factors, (iii) multiphasic solutions and (iv) multicompartmental systems. Thus a truly complete simulation of the absorption, transport, utilization and excretion of the trace elements would become possible. This is *the* computer application for bioinorganic chemists in the future.

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APPENDIX A1

Instructions and FORTRAN listing of

the computer program INDEX

INDEX

DATA PREPARATION FOR PROCESSING LITERATURE REFERENCES
BY COMPUTER USING PROGRAM INDEX

This document describes how the input data for program INDEX in the Bio-inorganic Chemistry computer library held by the South West Universities Computer Network should be punched onto data cards. It refers only to the way in which the data should be coded. A separate document entitled "The LITREF Macro" gives details of the Job Control commands required to call and execute the programs.

Introduction

Program INDEX is used to produce lists of literature references sorted according to one of the following: (i) the order in which they were entered, (ii) journal name, (iii) author name, (iv) keyword, (v) date, (vi) Chemical Abstract number, and (vii) a list of reference numbers provided by the user.

The input consists of alphanumeric strings which specify each author, journal, book and keyword in coded form. The year, journal volume and initial page number of each reference are also entered. In addition, there is the option to include the Chemical Abstract number of the reference and a user-designated alphanumeric string which can be used to indicate whether a photocopy has been procured and if so where it is filed.

Each author, journal, book title and keyword is assigned a sequence number. Except for the list of authors, these are common to all users and must be taken from current lists available from P.M.M. If a reference is not covered in this respect by the existing journal, book or keyword lists, these can be updated to include new entries by P.M.M. upon request.

The data for the LITREF Macro is divided into two sections. Details of each are provided below. They are separated in the input data file by one blank card. Both the author and the reference entries may be made in any order so that a literature database may be built up sequentially. However, the sequence numbers given by the user to both the authors and the references must be complete and in order.

Stage 1: The list of authors

This stage of the input data begins with a card reading 'AUTHOR LIST', beginning in column 1. This is immediately followed by a set of cards containing the sequence number and name of each author.

The first four columns on each card hold the sequence number as a left-justified integer. The next column is left blank. Columns 6-26 hold the author name as a right-justified alphanumeric string in which the surname appears first and the initials follow after a single space. Initials should each be separated by a full stop without the spacing.

For example:

```
AUTHOR LIST
0001 DICKENS C.
0002 DARWIN C.
0003 SHAKESPEARE W.
0004 POE E.A.
```

Stage 2: The list of references

This stage of the input data begins with a card reading 'REFERENCES', beginning in column 1. This is immediately followed by the list of references. Each comprises at least four cards.

1. The first card contains the following information:

   (a) The sequence number of the reference
   (b) The Chemical Abstract number (optional)
   (c) The journal number
   (d) The year
   (e) The volume number of the journal
   (f) The page number of the reference
   (g) The users identifying initials and a two-lettered filing code (the latter is optional)
   (h) The sequence numbers for up to 7 authors
   (i) An alphanumeric string that is to appear next to the page number (optional)

FORMAT (I4, I9, 4I5, 1X, A4, 7I5, 3X, A4)

Note that except for the Chemical Abstract number each item consists of 4 digits or characters. Thus the above format is such that all except the last are separated from one another by a blank space.

2. The second card contains a string of up to 20 keyword numbers. There must be at least one per reference. As these are of three digits each, the format is such that again each is separated from the previous one by a blank space. However, the first column must be left blank.

FORMAT (20I4)

3. The third and subsequent cards carry the title of the article. Up to six lines are permitted. All must be left-justified.

FORMAT (20A4)

4. One blank card to delimit the reference from the next one.

For example, the reference data could begin as follows.

```
REFERENCES
0001 72345678 0078 1975 0001 1234 PMXX 0001
 023 009 114 099
THE MISERLY SIDE OF MR.MICAWBER

0002          0001 1956 0101 0001 PMZZ 0003 0004
 185
AN INTERESTING STUDY BY TWO AUTHORS WHO WERE NOT EVEN
CONTEMPORARIES

0003 890000123 0111 1922 0122 0066 PMYY 0004 0002
 002
THE ORIGIN OF THE CROW SPECIES
```

The following idiosyncrasies may be useful to the advanced user.

1. Books are referenced by indicating a journal number of 0001. On such occasions the sequence number of the book is entered in place of the journal's volume number. For example, reference 0002 above refers to a book whose title has the sequence number 0101.

2. If the year is coded as 9999 then this figure is omitted whenever the reference is printed out.

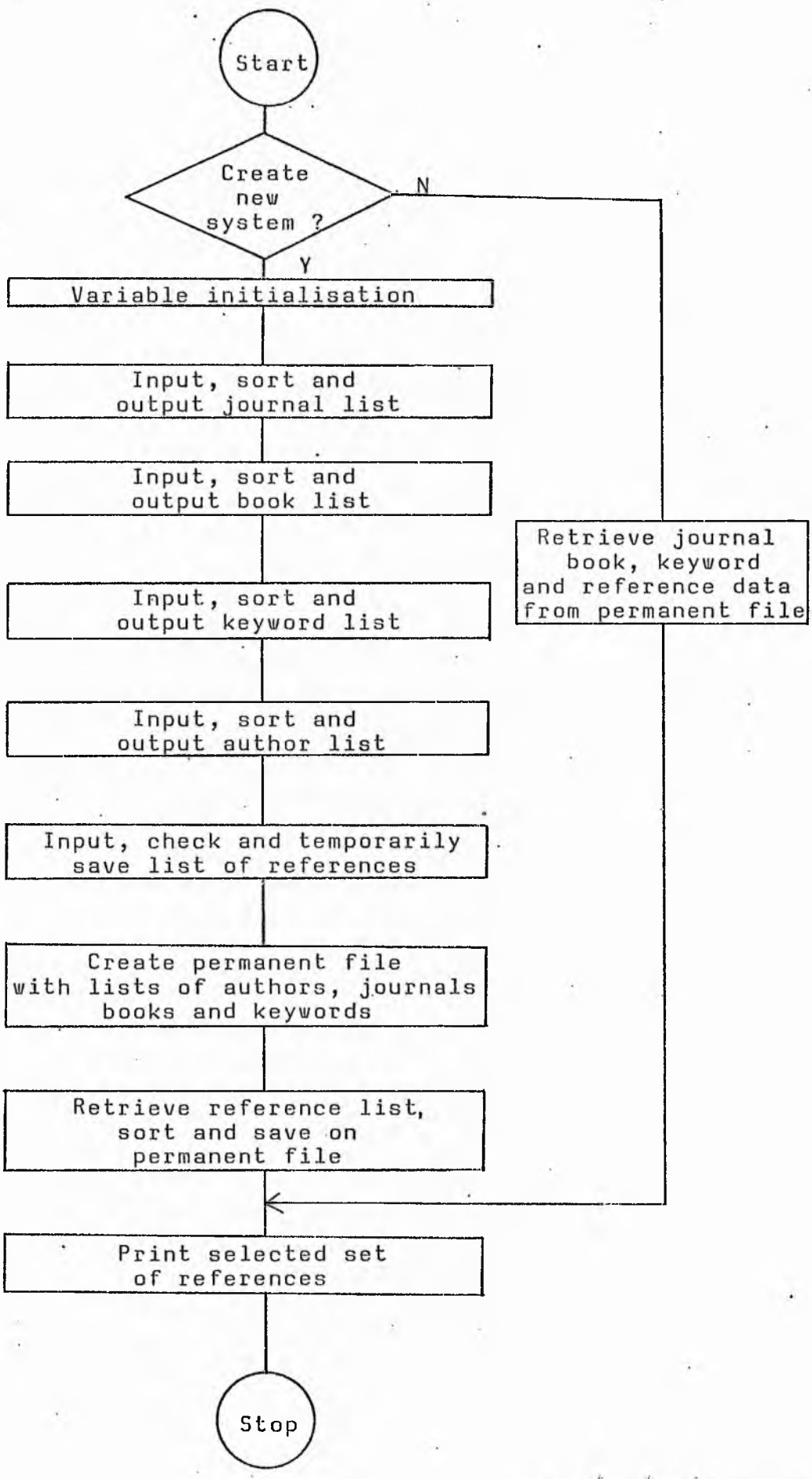
3. If the page number of a book is coded as 0000, the title card of the reference is not printed. Nevertheless, a dummy title must be supplied on input. By convention this is the single word 'BOOK'.

4. The final four character alphanumeric string on the first card of each reference is conventionally reserved for those journals which designate pages by a combination of letters and digits. The former can be appended to the page number by entering the appropriate letters in columns 77-80.

5. The two elements of a Chemical Abstract number should be separated by as many zeros as are required to fill the entire field of nine columns. Hence, 89:123 becomes 890000123 and 72:345678 becomes 72345678. The parity letter used by Chemical Abstracts is omitted.

6. Several options are available when the user specifies a list of numbers for those references that are required to be printed. Ordinarily, a list is produced in the order given but numbered sequentially from one onwards. A negative number entered as a dummy for the first reference will cause the list to be printed with the user's reference number and filing code rather than the sequential numbers described above. By specifying -999 as the dummy first reference, the list of numbers is treated as though it had been sorted according to the first named author of each reference. These facilities are useful when preparing publications for journals with different requirements concerning the format of references.

FLOW DIAGRAM FOR PROGRAM INDEX



```
C       PROGRAM INDEX.
C       --------------
C
C
C
C       THIS PROGRAM SETS UP AND OPERATES A LITERATURE
                  INDEX AND CATALOGUE
C
C       IT WAS DEVELOPED AT THE UNIVERSITY OF CAPE TOWN
                  DURING 1975
C       AND REWRITTEN AT THE UNIVERSITY OF ST. ANDREWS IN
                  1976.
C       IT WAS MODIFIED AT UWIST BETWEEN 1978 AND 1980.
C       LAST ALTERED ON 8/2/1980.
C       THE PROGRAM IS WRITTEN IN FORTRAN IV
C
        IMPLICIT INTEGER(A-Z)
        DIMENSION  REF(12,2000), REFA(2,5000), REFK(2,7000)
        DIMENSION  KEY(12,350), JURNAL(12,400),
                   BOOK(20,150), CM(2000),
       1  FC(2000), AUTHOR(7,2500), R(2500), S(2500),
                   T(2500)
        DIMENSION  NUMBER(20), LIT(11), LINE18(18),
                   LISTIT(500)
        DIMENSION  TIE1(2), TIE2(2), REFSRT(2000)
        LOGICAL  LABS, LKEY, NEW
        COMMON /ONE/  NLINES, MLINES, LABS, LKEY
        COMMON /TWO/  IN, OUT, FSEQ, FDEF
        COMMON /THREE/  LINE20(20), NUMBER(20), PAGE(10),
                   NEW
C
C
        EQUIVALENCE  (REF(1,1), AUTHOR(1,1)), (REFK(1,1),
                   R(1))
        EQUIVALENCE  (R(2500), TIE1(1)), (S(2500), TIE2(1))
        EQUIVALENCE  (S(1), TIE1(2)), (T(1), TIE2(2))
C       EQUIVALENCE  (REF(1,MR+1), FC(1)), (FC(MR+1), CM(1))
C
        DATA NUMBER/'1   2   3   4   5   6   7   8   9
                   10  11  12  13  1
       14  15  16  17  18  19  20'/
        DATA LIT/'    JOURBOOKKEYWAUTHET. AL.
                   CREACHROSELEREFE'/
C
C
C
C
 1000 FORMAT(I1,2X,A4,7X,A4)
 1001 FORMAT(20A4)
 1101 FORMAT(I4,I9,4I5,1X,A4,7I5,3I1,A4)
 1121 FORMAT(20I4)
 3121 FORMAT(///'0','ONLY SIX TITLE LINES ARE
                   PERMITTED.',/'0',
      1 'ERROR ON CARD NUMBER',I5,////)
 2211 FORMAT(2I4,10A4)
 2401 FORMAT(11I4,2A4,I8)
```

```
 3140 FORMAT(//'0','MISSING REFERENCE NUMBER',I6,//)
 3275 FORMAT('0',10X,A4,5X,20I5)
 3285 FORMAT(' ',10X,4(I8,' = ',I4,10X))
 3649 FORMAT('0',T30,20(1H*),5X,'INCORRECT KEYWORD NO. IN
                  REF',I5,/)
 3650 FORMAT('1','INDEX SYSTEM CREATED.',/'
                  ',20(1H*),///'0',T25,'NUMBER
     1 OF DATA CARDS =',I5,/' ',T25,'NUMBER OF REFERENCES
                  =',I5,/' ',
     2 T25,'NUMBER OF AUTHORS =',I5,/' ',T25,'NUMBER OF
                  KEYWORDS =',
     3 I5,/' ',T25,'NUMBER OF JOURNALS =',I4,/'
                  ',T25,'NUMBER OF BOOKS =
     4',I4,/' ',T25,'NUMBER OF AUTHOR ENTRIES =',I5,/'
                  ',T25,
     5'NUMBER OF KEYWORD ENTRIES =',I5,/' ',T25,
     6'NUMBER OF TITLE LINES =',I5,/' ',T25,
     7'SELECTOR CODE =',I5,//////)
 3810 FORMAT('0','ERROR TERMINATION',/' ','CODE',I6,/'
                  ','CARD',I6)
 3811 FORMAT('0','NJ =',I5,3X,'NB =',I5,3X,'NK
                  =',I5,3X,'NR =',I6,////)
 3961 FORMAT('1',///'0',5X,'KEY TO THE REFERENCE
                  NUMBERS',///'0',5X,
     1   2('REFERENCE',4X,'CATALOGUE',15X),/////)
 3965 FORMAT(' ',5X,2(I6,7X,I6,18X))
 3966 FORMAT(//'0','ERROR(S) IN REFERENCE LIST',/)
 3999 FORMAT(' ','SUCCESSFUL TERMINATION.',///)
 9999 FORMAT('1')
C
C
C      INITIALISATION.
C
C
       MR = 2000
       MA = 2500
       MJ = 400
       MK = 350
       MB = 150
       MRA = 5000
       MRK = 7000
C
       OUT = 6
       FSEQ = 2
       FTEMP = 3
       FDEF = 4
       NCARD = 5
       IN = NCARD
       MLINES = 1000000
       NJ = 0
       NB = 0
       NK = 0
       NR = 0
       NERR = 0
C
```

```
C
C
      DO 50 I=1,20
   50 NUMBER(I) = NUMBER(I)
      M = LIT(1)
      DO 51 J=1,MK
      DO 51 I=1,12
   51 KEY(I,J) = M
      DO 52 J=1,MJ
      DO 52 I=1,12
   52 JURNAL(I,J) = M
      DO 53 J=1,MB
      DO 53 I=1,20
   53 BOOK(I,J) = M
      DO 54 J=1,MA
      DO 54 I=1,7
   54 AUTHOR(I,J) = M
C
C
C
      READ(NCARD,1000)  J, I, K
      WRITE(OUT,10078)  J, I, K
10078 FORMAT('0',I5,5X,A4,5X,A4)
      NN = 1
      IF(J.NE.0)  IN = J
C
C     INFO -  NCAT=6;CREATE ONLY; NCAT=7;CREATE & LIST
C
      NCAT = 0
      IF(I.EQ.LIT(8))  NCAT = 7
      IF(I.EQ.LIT(8))  I = K
      IF(I.EQ.LIT(9))  NCAT = NCAT + 1
      IF(I.EQ.LIT(2))  NCAT = NCAT + 2
      IF(I.EQ.LIT(5))  NCAT = NCAT + 3
      IF(I.EQ.LIT(4))  NCAT = NCAT + 4
      IF(I.EQ.LIT(10)) NCAT = NCAT + 5
      IF(I.EQ.LIT(1).AND.K.EQ.LIT(1))  NCAT = 6
      IF(NCAT.EQ.0)  STOP  1000
      IF(NCAT.LT.6)  GO TO 600
C
C
C     CREATE THE INDEX
C
                                  CODE 1
C
      WRITE(OUT,9999)
      IR = 6
      NN = 2
      READ(IN,1001)  IXA
      IF(IXA.NE.LIT(2))  GO TO 801
      CALL NULIST(JURNAL,MJ,12,NJ,NN,IN,OUT,LINE20,R,S,T)
      IF(NJ.LE.0)  GO TO 801
      CALL LIST(JURNAL,12,NJ,OUT)
      WRITE(OUT,9999)
C
```

```
C
                                    CODE 2
C
      READ(IN,1001)  IXA
      NN = NN + 1
      IF(IXA.NE.LIT(3))  GO TO 802
      CALL NULIST(BOOK,MB,20,NB,NN,IN,OUT,LINE20,R,S,T)
      IF(NB.LE.0)  GO TO 802
      CALL LIST(BOOK,20,NB,OUT)
      WRITE(OUT,9999)
C
C
                                    CODE 3
C
      READ(IN,1001)  IXA
      NN = NN + 1
      IF(IXA.NE.LIT(4))  GO TO 803
      CALL NULIST(KEY,MK,12,NK,NN,IN,OUT,LINE20,R,S,T)
      IF(NK.LE.0)  GO TO 803
      CALL LIST(KEY,12,NK,OUT)
      WRITE(OUT,9999)
C
C
                                    CODE 4
C
      READ(IN,1001)  IXA
      NN = NN + 1
      IF(IXA.NE.LIT(5))  GO TO 804
      CALL NULIST(AUTHOR,MA,7,NA,NN,IN,OUT,LINE20,R,S,T)
      IF(NA.LE.0)  GO TO 804
      CALL LIST(AUTHOR,7,NA,OUT)
      WRITE(OUT,9999)
C
C
                                    CODE 5
C
      READ(IN,1001)  IXA
      NN = NN + 1
      IF(IXA.NE.LIT(11))  GO TO 805
      NRT = 1
      DO 126 NR=1,MR
      READ(IN,1101,ERR=805,END=130)  LINE18
      NN = NN + 1
      IF(LINE18(1).EQ.0)  GO TO 130
      IF(LINE18(1).NE.NR)  GO TO 140
      WRITE(FTEMP,1101)  LINE18
      J = LINE18(3)
      IF(J.GT.NJ.OR.J.LE.0)  GO TO 805
      R(NR) = (LINE18(4) * 10000) + JURNAL(1,J)
      S(NR) = (LINE18(5) * 10000) + LINE18(6)
      T(NR) = NR
      L = 1
      IA = LINE18(8)
      IF(IA.GT.NA.OR.IA.LE.0)  GO TO 805
      I = AUTHOR(2,IA) + 2
```

```
      DO 101 M=3,I
      LINE20(L) = AUTHOR(M,IA)
  101 L = L + 1
      LINE20(L) = LIT(1)
      L = L + 1
      IF(LINE18(16).NE.0.OR.LINE18(17).NE.0)  GO TO 110
      N = L
      DO 103 J=9,14
      IA = LINE18(J)
      IF(IA.EQ.0)  GO TO 115
      IF(IA.GT.NA.OR.IA.LE.0)  GO TO 805
      I = AUTHOR(2,IA) + 2
      DO 102 M=3,I
      IF(N.GT.20)  GO TO 110
      LINE20(N) = AUTHOR(M,IA)
  102 N = N + 1
      IF(N.GT.20)  GO TO 103
      LINE20(N) = LIT(1)
      N = N + 1
  103 CONTINUE
      GO TO 115
  110 LINE20(L) = LIT(6)
      L = L + 1
      LINE20(L) = LIT(7)
      N = L + 1
  115 IF(N.GT.20)  GO TO 120
      DO 116 M=N,20
  116 LINE20(M) = LIT(1)
C
C
C
  120 L = NRT
      WRITE(FDEF,REC=NRT)  LINE20
      NRT = NRT + 1
      NN = NN + 1
      READ(IN,1121,ERR=805)  LINE20
      WRITE(FTEMP,1121)  LINE20
      DO 121 I=1,6
      NN = NN + 1
      READ(IN,1001,END=125)  LINE20
      IF(LINE20(1).EQ.LIT(1))  GO TO 125
      WRITE(FDEF,REC=NRT)  LINE20
  121 NRT = NRT + 1
      WRITE(OUT,3121)  NN
      GO TO 815
  125 WRITE(FTEMP,1121)  I, L
      CM(NR) = LINE18(2)
  126 FC(NR) = LINE18(7)
      STOP 1126
  130 NR = NR - 1
      NRT = NRT + 1
      ENDFILE FTEMP
      GO TO 200
C
C
```

```
C
  140 WRITE(OUT,3140)  NR
      WRITE(OUT,3810)
      STOP 1140
C
C
C     BEGIN THE PERMANENT SEQUENTIAL FILE.
C     STORE AUTHOR, JOURNAL, BOOK AND KEYWORD LISTS.
C
C
  200 WRITE(FSEQ,1121)  NA, NJ, NB, NK
      DO 202 POS=1,NA
      DO 201 J=1,NA
      IF(AUTHOR(1,J).EQ.POS)  GO TO 202
  201 CONTINUE
      STOP 1201
  202 WRITE(FSEQ,2211)  J, (AUTHOR(I,J), I=2,7)
C
C
C
      IF(NCAT.GT.7)  GO TO 250
      DO 211 J=1,NJ
  211 WRITE(FSEQ,2211)  (JURNAL(I,J), I=1,12)
      DO 213 J=1,NB
      WRITE(FSEQ,1121)  BOOK(1,J), BOOK(2,J)
  213 WRITE(FSEQ,1001)  (BOOK(I,J), I=3,20)
      DO 216 J=1,NK
  216 WRITE(FSEQ,2211)  (KEY(I,J), I=1,12)
C
C
C     SORT THE REFERENCES AND STORE THE RESULT IN REFSRT.
C
C
  250 CALL ORDER(R,S,T,NR)
      DO 251 J=1,NR
  251 REFSRT(J) = T(J)
C
C
C     OUTPUT FILING CODE AND CHEMICAL ABSTRACT NUMBER
                LISTS.
C
C
      DO 271 IR=1,NR
      R(IR) = FC(IR)
  271 S(IR) = IR
      CALL ORDER(R,S,T,NR)
      M = 1
      J = 0
      DO 275 IR=1,NR
      IF(R(IR).EQ.R(M).AND.J.LT.20)  GO TO 274
      WRITE(OUT,3275)  R(M), (LINE20(I), I=1,J)
      J = 0
      M = IR
  274 J = J + 1
  275 LINE20(J) = S(IR)
```

```
      WRITE(OUT,3275)  R(M), (LINE20(I), I=1,J)
      WRITE(OUT,9999)
C
C
C
      J = 0
      DO 281 IR=1,NR
      IF(CM(IR).LT.25000000)  GO TO 281
      J = J + 1
      R(J) = CM(IR)
      S(J) = IR
  281 CONTINUE
      IF(J.LT.10)  GO TO 300
      CALL ORDER(R,S,T,J)
      L = (J / 4) * 4
      DO 285 I=1,L,4
      K = I + 3
  285 WRITE(OUT,3285)  (R(M), S(M), M=I,K)
      L = L + 1
      IF(L.GT.J)  GO TO 300
      WRITE(OUT,3285)  (R(M), S(M), M=L,J)
      WRITE(OUT,9999)
C
C
C     LOAD THE REFERENCES
C
C
  300 IRA = 1
      IRK = 1
      REWIND FTEMP
      DO 310 IR=1,NR
      READ(FTEMP,1101)  LINE18
      REF(1,IR) = LINE18(1)
      REF(2,IR) = LINE18(3)
      REF(3,IR) = LINE18(4)
      REF(4,IR) = LINE18(5)
      REF(5,IR) = LINE18(6)
      REF(11,IR) = IRA
      REF(12,IR) = LINE18(18)
      DO 301 J=8,18
      IF(LINE18(J).EQ.0)  GO TO 302
      IF(IRA.GT.MRA)  STOP 1301
      REFA(1,IRA) = LINE18(J)
  301 IRA = IRA + 1
      STOP 1302
  302 REF(10,IR) = J - 8
      READ(FTEMP,1121)  LINE20
      REF(7,IR) = IRK
      DO 305 J=1,20
      IF(LINE20(J).EQ.0)  GO TO 306
      IF(IRK.GT.MRK)  STOP 1304
      REFK(1,IRK) = LINE20(J)
  305 IRK = IRK + 1
      J = 21
  306 REF(6,IR) = J - 1
```

```
  310 READ(FTEMP,1121)  REF(8,IR), REF(9,IR)
      NRA = IRA - 1
      NRK = IRK - 1
C
C
C     PRINT OUT THE REFERENCES IN THE ORDER THEY WERE
                ENTERED.
C
C
  350 NLINES = 0
      IF(NCAT.NE.7)  GO TO 400
      WRITE(OUT,9999)
      NEW = .FALSE.
      LABS = .FALSE.
      LKEY = .FALSE.
      DO 351 J=1,10
  351 PAGE(J) = LIT(1)
      DO 352 IR=1,NR
  352 CALL ROUT(REF,CM,FC,KEY,REFK,BOOK,JURNAL,MR,MK,MRK,
                MB,MJ,IR)
C
C
C     OUTPUT REFERENCES TO TEMPORARY FILE;
                RELOAD IN SORTED ORDER.
C
C
  400 REWIND FTEMP
      DO 401 J=1,NR
      IR = REFSRT(J)
      WRITE(FTEMP,2401)  (REF(I,IR), I=1,12), FC(IR),
                CM(IR)
      K = REF(7,IR)
      L = K + REF(6,IR) - 1
      WRITE(FTEMP,1121)  (REFK(1,I), I=K,L)
      K = REF(11,IR)
      L = REF(10,IR) + K - 1
  401 WRITE(FTEMP,1121)  (REFA(1,I), I=K,L)
      ENDFILE FTEMP
      REWIND FTEMP
      IRA = 1
      IRK = 1
  450 DO 453 IR=1,NR
      READ(FTEMP,2401)  (REF(I,IR), I=1,12), FC(IR),
                CM(IR)
      REF(7,IR) = IRK
      L = IRK + REF(6,IR) - 1
      READ(FTEMP,1121)  (REFK(1,I), I=IRK,L)
      DO 451 I=IRK,L
  451 REFK(2,I) = IR
      IRK = L + 1
      REF(11,IR) = IRA
      L = IRA + REF(10,IR) - 1
      READ(FTEMP,1121)  (REFA(1,I), I=IRA,L)
      DO 452 I=IRA,L
  452 REFA(2,I) = IR
```

```
  453 IRA = L + 1
      NRK = IRK - 1
      NRA = IRA - 1
      REWIND FTEMP
C
C
C     STORE THE ARRAYS REF, FC, CM, REFA, REFK ON
                PERMANENT FILE.
C
C
  500 IF(NCAT.GT.7)  GO TO 510
      WRITE(FSEQ,1121)  NR, NRA, NRK, NRT
      DO 501 IR=1,NR
  501 WRITE(FSEQ,2401)  (REF(I,IR), I=1,11), FC(IR),
                    CM(IR)
      DO 502 IRA=1,NRA
  502 WRITE(FSEQ,2401)  REFA(1,IRA), REFA(2,IRA)
      DO 503 IRK=1,NRK
  503 WRITE(FSEQ,2401)  REFK(1,IRK), REFK(2,IRK)
C
  510 ENDFILE FSEQ
      REWIND FSEQ
      GO TO 650
C
C
C     LOAD ARRAYS FROM THE PERMANENT SEQUENTIAL FILE.
C
C
  600 READ(FSEQ,1121)  NA, NJ, NB, NK
      DO 601 IA=1,NA
  601 READ(FSEQ,1121)  J
      DO 602 J=1,NJ
  602 READ(FSEQ,2211)  (JURNAL(I,J), I=1,12)
      DO 603 J=1,NB
      READ(FSEQ,1121)  BOOK(1,J), BOOK(2,J)
  603 READ(FSEQ,1001)  (BOOK(I,J), I=3,20)
      DO 604 J=1,NK
  604 READ(FSEQ,2211)  (KEY(I,J), I=1,12)
      READ(FSEQ,1121)  NR, NRA, NRK, NRT
      DO 610 IR=1,NR
  610 READ(FSEQ,2401)  (REF(I,IR), I=1,11), FC(IR), CM(IR)
      DO 612 IRA=1,NRA
  612 READ(FSEQ,2401)  REFA(1,IRA), REFA(2,IRA)
      DO 613 IRK=1,NRK
      READ(FSEQ,2401)  REFK(1,IRK), REFK(2,IRK)
      J = REFK(1,IRK)
      IF(J.GT.0.AND.J.LE.MK)  GO TO 613
      WRITE(OUT,3649)  REFK(2,IRK)
      REFK(1,IRK) = 1
  613 CONTINUE
      REWIND FSEQ
  650 WRITE(OUT,3650)  NN, NR, NA, NK, NJ, NB, NRA, NRK,
                    NRT, NCAT
      WRITE(OUT,9999)
      GO TO 900
```

```
C
C
C       ABORT THE RUN
C
C
  801 IR = IR - 1
  802 IR = IR - 1
  803 IR = IR - 1
  804 IR = IR - 1
  805 IR = IR - 1
  810 WRITE(OUT,3810)  IR, NN
  815 WRITE(OUT,3811)  NJ, NB, NK, NR
      NERR = 1
      GO TO 999
C
C
C       SELECT THE REFERENCES FOR THE CURRENT CATALOGUE.
C
  900 NLINES = 0
      LABS = .FALSE.
      LKEY = .FALSE.
      DO 901 I=1,10
  901 PAGE(1) = LIT(1)
      IF(NCAT.GT.7)  NCAT = NCAT - 7
      GO TO (910,920,930,940,950,999,999),  NCAT
C
C       CHRONOLOGICAL CATALOGUE.
C
  910 NEW = .FALSE.
      DO 912 IR=1,NR
  912 CALL ROUT(REF,CM,FC,KEY,REFK,BOOK,JURNAL,MR,MK,MRK,
                  MB,MJ,IR)
      GO TO 999
C
C       JOURNAL CATALOGUE.
C
  920 POS = 1
      LKEY = .TRUE.
      LABS = .TRUE.
  921 DO 922 J=1,NJ
      IF(JURNAL(1,J).EQ.POS)  GO TO 923
  922 CONTINUE
      GO TO 927
  923 NEW = .TRUE.
      L = 3
      PAGE(1) = LIT(1)
      PAGE(2) = LIT(1)
      IF(JURNAL(2,J).GT.8)  L = 11 - JURNAL(2,J)
      K = 3
      DO 924 I=L,10
      PAGE(I) = JURNAL(K,J)
  924 K = K + 1
      L = 1
  925 DO 926 IR=L,NR
      IF(REF(2,IR).EQ.J)  GO TO 928
```

```
  926 CONTINUE
  927 IF(POS.EQ.NJ)  GO TO 999
      POS = POS + 1
      GO TO 921
  928 CALL ROUT(REF,CM,FC,KEY,REFK,BOOK,JURNAL,MR,MK,MRK,
                MB,MJ,IR)
      IF(L.EQ.NR)  GO TO 927
      L = IR + 1
      GO TO 925
C
C     AUTHOR CATALOGUE
C
  930 POS = 1
      READ(FSEQ,1121)  I, J, K, L
      PAGE(1) = LIT(1)
      PAGE(2) = LIT(1)
      PAGE(8) = LIT(1)
      PAGE(9) = LIT(1)
      PAGE(10) = LIT(1)
  931 NEW = .TRUE.
      READ(FSEQ,2211)  I, J, (PAGE(L), L=3,7)
      L = 1
  932 DO 933 IRA=L,NRA
      IF(REFA(1,IRA).EQ.I)  GO TO 935
  933 CONTINUE
  934 IF(POS.EQ.NA)  GO TO 936
      POS = POS + 1
      GO TO 931
  935 IR = REFA(2,IRA)
      CALL ROUT(REF,CM,FC,KEY,REFK,BOOK,JURNAL,MR,MK,MRK,
                MB,MJ,IR)
      IF(L.EQ.NRA)  GO TO 934
      L = IRA + 1
      GO TO 932
  936 REWIND FSEQ
      GO TO 999
C
C     KEYWORD CATALOGUE
C
  940 POS = 1
  941 DO 942 K=1,NK
      IF(KEY(1,K).EQ.POS)  GO TO 943
  942 CONTINUE
      GO TO 947
  943 NEW = .TRUE.
      L = 3
      PAGE(1) = LIT(1)
      PAGE(2) = LIT(1)
      IF(KEY(2,K).GT.8)  L = 11 - KEY(2,K)
      J = 3
      DO 944 I=L,10
      PAGE(I) = KEY(J,K)
  944 J = J + 1
      L = 1
  945 DO 946 IRK=L,NRK
```

```
      IF(REFK(1,IRK).EQ.K)  GO TO 948
  946 CONTINUE
  947 IF(POS.EQ.NK)  GO TO 999
      POS = POS + 1
      GO TO 941
  948 IR = REFK(2,IRK)
      CALL ROUT(REF,CM,FC,KEY,REFK,BOOK,JURNAL,MR,MK,MRK,
     1          MB,MJ,IR)
      IF(L.EQ.NRK)  GO TO 947
      L = IRK + 1
      GO TO 945
C
C     LIST SELECTED REFERENCES.
C
  950 L = 0
      NEW = .FALSE.
      ILIST = 0
      K = 0
  951 L = L + 1
  952 READ(IN,1121,END=957,ERR=951)  POS
      IF(POS.GT.NR)  GO TO 951
      IF(POS.LE.0)  GO TO 958
      ILIST = ILIST + 1
      IF(ILIST.GT.500) GO TO 954
      LISTIT(ILIST) = POS
      IF(ILIST.EQ.1.OR.K.EQ.9)  GO TO 954
      ILIST = ILIST - 1
      DO 953 IR=1,ILIST
      IF(POS.EQ.LISTIT(IR))  GO TO 952
  953 CONTINUE
      ILIST = ILIST + 1
  954 IF(K.EQ.1)  GO TO 951
      DO 955 IR=1,NR
      IF(REF(1,IR).EQ.POS)  GO TO 956
  955 CONTINUE
      GO TO 951
  956 J = REF(1,IR)
      IRA = FC(IR)
      IF(K.NE.9)  REF(1,IR) = L
      FC(IR) = LIT(1)
      CALL ROUT(REF,CM,FC,KEY,REFK,BOOK,JURNAL,MR,MK,MRK,
     1          MB,MJ,IR)
      REF(1,IR) = J
      FC(IR) = IRA
      IF(K.EQ.2)  GO TO 959
      GO TO 951
  957 IF(K.NE.1)  GO TO 960
      K = 2
C
C     SORT BY AUTHOR, IF REQUIRED
C
      I = 0
      DO 971 IR=1,NR
      L = 0
  970 L = L + 1
```

```
      IF(L.GT.ILIST)  GO TO 971
      IF(REF(1,IR).NE.LISTIT(L))  GO TO 970
      I = I + 1
      CM(I) = REF(11,IR)
  971 CONTINUE
      READ(FSEQ,1121)  I
      I = 0
      DO 974 IA=1,NA
      READ(FSEQ,2211)  J
      L = 1
  972 IRA = CM(L)
      IF(REFA(1,IRA).NE.J)  GO TO 973
      I = I + 1
      LISTIT(I) = REFA(2,IRA)
      IR = LISTIT(I)
  973 L = L + 1
      IF(L.LE.ILIST)  GO TO 972
  974 CONTINUE
C
      L = 0
      GO TO 959
  958 IF(L.NE.1)  GO TO 951
      K = 9
      IF(POS.EQ.-999)  K = 1
      GO TO 952
  959 IF(L+1.GT.ILIST)  GO TO 960
      L = L + 1
      IR = LISTIT(L)
      LISTIT(L) = REF(1,IR)
      GO TO 956
  960 DO 961 IR=1,ILIST
      R(IR) = LISTIT(IR)
      S(IR) = LISTIT(IR)
  961 T(IR) = IR
      CALL ORDER(R,S,T,ILIST)
      WRITE(OUT,3961)
      DO 965 IR=1,ILIST
  965 WRITE(OUT,3965)  IR, LISTIT(IR), T(IR), R(IR)
      IF(L.NE.ILIST)  WRITE(OUT,3966)
C
C     END
C
  999 IF(NCAT.NE.6)  WRITE(OUT,9999)
      IF(NERR.EQ.0)  WRITE(OUT,3999)
      STOP
      END
-------------------------------------------------------------------
      SUBROUTINE LIST(ARRAY,NW,NN,OUT)
C
C
C
C     THIS SUBROUTINE PRINTS OUT THE AUTHOR, JOURNAL,
                  BOOK AND KEYWORD
C     LISTS IN BOTH THEIR ENTERED AND ALPHABETICALLY
                  SORTED ORDER.
C
```

```
C
C
      IMPLICIT INTEGER(A-Z).
      DIMENSION  ARRAY(NW,NN)
C
C
C
 3130 FORMAT(' ',I5,5X,5A4,24X,I5,5X,5A4)
 3140 FORMAT(' ',I5,5X,10A4,4X,I5,5X,10A4)
 3150 FORMAT(' ',2I5,5X,20A4)
C
C
C
      DO 160 POS=1,NN
      DO 110 J=1,NN
      IF(ARRAY(1,J).EQ.POS) GO TO 120
  110 CONTINUE
      STOP 41
  120 K = ARRAY(2,J) + 2
      IF(NW-12) 130,140,150
  130 WRITE(OUT,3130)  POS,(ARRAY(I,POS), I=3,7),
                  J,(ARRAY(I,J), I=3,K)
      GO TO 160
  140 WRITE(OUT,3140)  POS,(ARRAY(I,POS),I=3,12),
                  J,(ARRAY(I,J),I=3,K)
      GO TO 160
  150 WRITE(OUT,3150)  POS, J, (ARRAY(I,J), I=3,K)
  160 CONTINUE
      RETURN
      END
```

```
      SUBROUTINE NULIST(ARRAY,MARR,MARRW,NARR,NN,IN,OUT,
                  LINE20,R,S,T)
C
C
C
C     THIS SUBROUTINE INPUTS THE AUTHOR, JOURNAL, BOOK
                  AND KEYWORD LISTS
C     FROM DATA CARDS.
C
C
C
      IMPLICIT INTEGER(A-Z)
      DIMENSION ARRAY(MARRW,MARR), LINE20(20), R(MARR),
                  S(MARR), T(MARR)
      DIMENSION  V(10), U(10), W(10)
      DATA  LIT/'    '/
C
C
C
 1011 FORMAT(I4,1X,18A4,A3)
 1121 FORMAT(A4)
 3122 FORMAT(//'0','THE PROGRAM LIMITS HAVE BEEN
                  EXCEEDED.')
C
C
```

```
C
      DO 120 NARR=1,MARR
      READ(IN,1011)  LINE20
      NN = NN + 1
      IF(LINE20(1).EQ.0)  GO TO 140
      IF(LINE20(1).NE.NARR)  GO TO 131
      DO 110 IARR=3,MARRW
      J = IARR - 1
      M = J
      IF(J.LE.10)  M = M + 1
      IF(LINE20(J).EQ.LIT.AND.LINE20(M).EQ.LIT)  GO TO 120
  110 ARRAY(IARR,NARR) = LINE20(J)
      J = J + 1
  120 ARRAY(2,NARR) = J - 2
      READ(IN,1121)  J
      IF(J.EQ.LIT)  GO TO 200
      WRITE(OUT,3122)
  130 NARR = 0
  131 NARR = -NARR
      RETURN
  140 NARR = NARR - 1
C
C     BEGIN SORT ON THE FIRST EIGHT CHARACTERS.
C
  200 DO 210 IARR=1,NARR
      R(IARR) = ARRAY(3,IARR)
      S(IARR) = ARRAY(4,IARR)
  210 T(IARR) = IARR
      CALL ORDER(R,S,T,NARR)
      DO 220 IARR=1,NARR
      J = T(IARR)
  220 ARRAY(1,J) = IARR
C
C     THE SORT IS NOW CONTINUED WITH THOSE ENTRIES WHOSE
                  FIRST EIGHT
C     CHARACTERS ARE IDENTICAL.
C
      M = 0
      DO 370 IARR=2,NARR
      IF(IARR.LE.M)  GO TO 370
      M = IARR
      J = IARR - 1
      IF(S(IARR).NE.S(J))  GO TO 370
      IF(R(IARR).NE.R(J))  GO TO 370
C
C
C
      DO 310 K=IARR,NARR
      IF(S(IARR).NE.S(K))  GO TO 320
  310 CONTINUE
      K = K + 1
C
C     POSITIONS J TO K ARE UNSORTED.
C
  320 K = K - 1
```

```
      IF(K.GT.IARR)  GO TO 340
C
C     J AND IARR ARE ADJACENT ....
C
      I = T(J)
      K = T(IARR)
      IF(ARRAY(5,I).LT.ARRAY(5,K))  GO TO 370
      IF(ARRAY(5,I).GT.ARRAY(5,K))  GO TO 330
      IF(ARRAY(6,I).LE.ARRAY(6,K))  GO TO 370
C
C     ..... AND INCORRECTLY POSITIONED.   SWOP THEM.
C
  330 ARRAY(1,I)  = IARR
      ARRAY(1,K)  = J
      GO TO 370
C
C      THERE ARE THREE OR MORE UNSORTED SEQUENTIAL VALUES.
C
  340 L = 0
      DO 350 I=J,K
      L = L + 1
      M = T(I)
      U(L) = ARRAY(5,M)
      V(L) = ARRAY(6,M)
  350 W(L) = M
      CALL ORDER(U,V,W,L)
      J = J - 1
      DO 360 I=1,L
      M = W(I)
  360 ARRAY(1,M) = I + J
      M = K
  370 CONTINUE
      RETURN
      END
      SUBROUTINE ORDER(R,S,T,N)
C
C
C
C     THIS ROUTINE SORTS THE VALUES IN THE THREE ARGUMENT
                ARRAYS.  THE
C     PRIMARY SORT IS BASED ON THE FIRST ARRAY VALUE BUT
                WHENEVER TWO OR
C     MORE OF THESE ARE IDENTICAL THE ORDER IS BASED ON
                THE VALUE IN THE
C     SECOND ARRAY.   THE THIRD ARRAY SIMPLY TAGS ALONG
                AND IS USED TO
C     HOLD THE ORIGINAL POSITION OF THE SORTED NUMBER
                PAIRS.
C
C
C
      IMPLICIT INTEGER(A-Z)
      DIMENSION R(N), S(N), T(N)
C
C
```

```
C
      M = 2
      DO 1 J=1,N
      M = M + M
      IF(M.GT.N)  GO TO 2
    1 CONTINUE
    2 M = M - 1
    3 N = (M - 1) / 2
      NN = N - M
      DO 8 I=1,NN
      OLD = I + M
      LR = R(OLD)
      LS = S(OLD)
      LT = T(OLD)
      DO 6 J=1,I,M
      NEW = OLD - M
C     IF(LR-R(NEW)) 5,4,7
C   4 IF(LS-S(NEW)) 5,7,7
      IF(LR.LT.R(NEW))  GO TO 5
      IF(LR.GT.R(NEW).OR.LS.GE.S(NEW))  GO TO 7
    5 R(OLD) = R(NEW)
      S(OLD) = S(NEW)
      T(OLD) = T(NEW)
      OLD = NEW
    6 CONTINUE
    7 R(OLD) = LR
      S(OLD) = LS
      T(OLD) = LT
    8 CONTINUE
      IF(M.GT.1)  GO TO 3
      RETURN
      END
```

```
      FUNCTION LENGTH(ARGN)
C
C
C
C     THIS FUNCTION RETURNS THE NUMBER OF DIGITS REQUIRED
                  BY THE VARIABLE
C     FORMAT STATEMENT TO OUTPUT THE ARGUMENT IN QUESTION.
C
C
C
      IMPLICIT INTEGER(A-Z)
      NUMBER = ARGN
      DO 100 I=1,11
      IF(NUMBER.LE.0)  GO TO 200
  100 NUMBER = NUMBER / 10
      STOP 81
  200 LENGTH = I - 1
      IF(LENGTH.LE.0)  LENGTH = 1
      RETURN
      END
```

```
      SUBROUTINE ROUT(REF,CM,FC,KEY,REFK,DOOK,JURNAL,MR,
                  NK,MRK,MB,MJ,IR)
C
```

```
C
C      THIS ROUTINE PRINTS OUT REFERENCE IR.
C
C
       IMPLICIT INTEGER (A-Z)
       DIMENSION  REF(12,MR), HOLD(2,20), REFK(2,MRK)
       DIMENSION  KEY(12,MK), JURNAL(12,MJ), VORMAT(14),
                  LIT(4), CM(MR),
     1 FC(MR), BOOK(20,MB)
       LOGICAL  LABS, LKEY, NEW
C
       COMMON /ONE/  NLINES, MLINES, LABS, LKEY
       COMMON /TWO/  IN, OUT, FSEQ, FDEF
       COMMON /THREE/  LINE20(20),  NUMBER(20), PAGE(10),
                  NEW
       DATA  LIT/'    ',',1H,',' ,  ',',1H,,'/
       DATA  VORMAT/'(1H ',',8X,',' ',',A4,',',2X,',',I4,',',
                  '1H,,',',2X,I',
     1   ' ',',1H,',',2X,',','I',' ',')'/
C
C
C
 3101  FORMAT(' ',92(1H*),10A4,/)
 3153  FORMAT('1',92X,10A4,/)
 3201  FORMAT(' ',I4,4X,20A4)
 3301  FORMAT(' ',A4,4X,20A4)
 3303  FORMAT(' ',8X,20A4)
 3601  FORMAT(' ',8X,'CHEMICAL ABSTRACT NUMBER',I4,':',I6)
C
C
C      LOCATE THE DEFINE FILE AUTHOR STRING.
C
  100  KSW = 2
       IRT = REF(9,IR)
C      FIND(FDEF'IRT)
C
C      IF NECESSARY, OUTPUT NEW HEADING
C
       IF(NLINES+12.GT.MLINES)  GO TO 140
       IF(.NOT.NEW)  GO TO 200
  101  NEW = .FALSE.
       WRITE(OUT,3101)  PAGE
       NLINES = NLINES + 2
       GO TO 200
C
C      CALCULATE THE NUMBER OF LINES NEEDED.
C
  140  LINES = REF(8,IR)
       IF(LABS.AND.CM(IR).GE.25000000)  LINES = LINES + 1
       IF(.NOT.LKEY)  GO TO 153
       KSW = 1
  150  IRK = REF(7,IR)
       NHK = REF(6,IR)
       DO 151 J=1,NHK
       IK = REFK(1,IRK)
```

```
      HOLD(1,J) = IK
      HOLD(2,J) = KEY(2,IK)
  151 IRK = IRK + 1
      IF(KSW.EQ.2)  GO TO 501
  152 CALL LOADK(HOLD,NHK,LINE20,KEY,MK,LIT(1),KSW)
      IF(KSW.EQ.0)  GO TO 153
      LINES = LINES + 1
      GO TO 152
C
C     PAGE, IF NECESSARY.
C
  153 IF(LINES+NLINES.LE.MLINES)  GO TO 154
      NLINES = 1
      IF(.NOT.NEW)  WRITE(OUT,3153)  PAGE
      IF(.NOT.NEW)  NLINES = NLINES + 1
      IF(NEW)  WRITE(OUT,3153)
  154 IF(NEW)  GO TO 101
C
C     PRINT OUT AUTHOR STRING.
C
  200 READ(FDEF,REC=IRT)  LINE20
      IRT = IRT + 1
C     FIND(FDEF'IRT)
      WRITE(OUT,3201)  REF(1,IR), LINE20
      NLINES = NLINES + 1
      IJ = REF(2,IR)
      IB = REF(4,IR)
      J = REF(5,IR)
      M = LENGTH(J)
      IF(IJ.EQ.1.AND.J.EQ.0)  GO TO 402
C
C     PRINT OUT TITLE STRINGS.
C
  300 NRT = IRT + REF(8,IR) - 2
      READ(FDEF,REC=IRT)  LINE20
      NLINES = NLINES + 1
      WRITE(OUT,3301)  FC(IR), LINE20
  301 IRT = IRT + 1
      IF(IRT-NRT)  302, 303, 400
  302 CONTINUE
C 302 FIND(FDEF'IRT)
  303 NLINES = NLINES + 1
      READ(FDEF,REC=IRT)  LINE20
      WRITE(OUT,3303)  LINE20
      GO TO 301
C
C     PRINT OUT THE JOURNAL OR BOOK REFERENCE.
C
  400 IF(IJ.EQ.1)  GO TO 402
      K = JURNAL(2,IJ)
      VORMAT(3) = NUMBER(K)
      IF(REF(3,IR).GT.3000.OR.REF(3,IR).LT.1000)
                  GO TO 408
      IF(IB.EQ.0)  GO TO 403
      VORMAT(13) = NUMBER(M)
```

```
      M = LENGTH(IB)
      VORMAT(9) = NUMBER(M)
      VORMAT(10) = LIT(2)
      K = K + 2
      WRITE(OUT,VORMAT)  (JURNAL(L,IJ), L=3,K),
                         REF(3,IR), IB, J
      GO TO 500
  402 K = BOOK(2,IB)
      VORMAT(3) = NUMBER(K)
      IF(REF(3,IR).GT.3000.OR.REF(3,IR).LT.1000)
                         GO TO 408
  403 K = K + 2
      VORMAT(9) = NUMBER(M)
      VORMAT(10) = LIT(1)
      VORMAT(13) = NUMBER(10)
      IF(IB.EQ.0)  GO TO 406
      IF(J.EQ.0)  GO TO 405
      WRITE(OUT,VORMAT)  (BOOK(L,IB), L=3,K), REF(3,IR), J
      GO TO 500
  405 VORMAT(7) = LIT(1)
      WRITE(OUT,VORMAT)  (BOOK(L,IB), L=3,K), REF(3,IR)
      VORMAT(7) = LIT(4)
      GO TO 500
  406 WRITE(OUT,VORMAT)  (JURNAL(L,IJ), L=3,K),
                         REF(3,IR), J
      GO TO 500
  408 K = K + 2
      IF(IJ.EQ.1)  WRITE(OUT,VORMAT)  (BOOK(L,IB), L=3,K)
      IF(IJ.NE.1)  WRITE(OUT,VORMAT)  (JURNAL(L,IJ),
                         L=3,K)
C
C     PRINT KEYWORD STRINGS
C
  500 NLINES = NLINES + 1
      IF(.NOT.LKEY)  GO TO 600
      IF(KSW.EQ.2)  GO TO 150
      KSW = 2
  501 CALL LOADK(HOLD,NHK,LINE20,KEY,MK,LIT(1),KSW)
      IF(KSW.EQ.0)  GO TO 600
      WRITE(OUT,3303)  LINE20
      NLINES = NLINES + 1
      GO TO 501
C
C     PRINT CHEMICAL ABSTRACT NUMBER.
C
  600 IF(.NOT.LABS.OR.CN(IR).LT.25000000)  GO TO 700
      I = CN(IR) / 1000000
      J = CN(IR) - (I * 1000000)
      WRITE(OUT,3601)  I, J
      NLINES = NLINES + 1
C
C     SPACE ONE LINE
C
  700 WRITE(OUT,3301)
      NLINES = NLINES + 1
```

```
      RETURN
      END
      SUBROUTINE LOADK(HOLD,NHK,LINE20,KEY,MK,LIT,KSW)
C
C
C     THIS ROUTINE OPTIMISES THE INSERTION OF KEYWORDS
                INTO ARRAY LINE20
C
C
      IMPLICIT INTEGER(A-Z)
      DIMENSION HOLD(2,20)
      DIMENSION LINE20(20), KEY(12,MK)
C
C
C
      NL = 1
  100 L = 0
      DO 110 I=1,NHK
      N = HOLD(2,I)
      IF(HOLD(1,I).LT.0.OR.N.LE.L)  GO TO 110
      IF(N+NL.GT.21)  GO TO 110
      IHK = I
      L = N
  110 CONTINUE
C
C
C
  112 IF(L.EQ.0)  GO TO 117
      J = HOLD(1,IHK)
      HOLD(1,IHK) = -J
      IF(KSW.EQ.1)  GO TO 116
      L = 3
      N = HOLD(2,IHK)
      DO 115 I=1,N
      LINE20(NL) = KEY(L,J)
      NL = NL + 1
  115 L = L + 1
  116 IF(NL.GT.20)  GO TO 117
      LINE20(NL) = LIT
      NL = NL + 1
      GO TO 100
C
C
C
  117 IF(NL.EQ.1)  GO TO 130
      IF(KSW.EQ.1.OR.NL.GT.20)  RETURN
      DO 118 I=NL,20
  118 LINE20(I) = LIT
      RETURN
C
C
C
  130 DO 131 I=1,NHK
  131 HOLD(1,I) = -HOLD(1,I)
      KSW = 0
      RETURN
      END
```

APPENDIX A2

Formation constant database for binary complex species used in the computer simulations

The following list of formation constants is used as the database for program MIX and program ECCLES. The values have been selected using the criteria stated in Section 2.1.3 (p.24). They are measured experimentally under the temperature and ionic strength of blood plasma (37C; 150 mmol dm^{-3}) or have been adjusted to conform to these conditions. The source of each value is indicated by one of the following symbols:

M - measured experimentally under model conditions

A - estimated from more than one measurement (taking averages where possible)

E - estimated from a single (i.e. unconfirmed) experimental determination under conditions different to those of the model

G - estimated from various chemical trends in the absence of an experimentally measured value

The main literature sources of the formation constants in the computer database are as follows.

"Stability Constants" (Special Publications 17 and 25) compiled by L.G.Sillen and A.E.Martell, 1964 and 1971, The Chemical Society, London

"Stability Constants of Metal-Ion Complexes", Part B (Organic Ligands) compiled by D.D.Perrin, 1979, Pergamon, Oxford

"Critical Stability Constants", Volumes 1-4, compiled by A.E.Martell and R.M.Smith, 1974-1976, Plenum Press, New York.

In addition, values from the following references are included.

26, 41, 48, 51, 52, 53, 56, 58, 59, 60, 61, 62, 63, 66, 67, 68, 69, 77, 79, 81, 108, 120, 121, 183, 192, 197, 214, 235, 243, 244, 249, 251, 252, 253, 254, 255, 256, 270, 271, 273, 276, 277, 279, 282, 287, 288, 306, 311, 312, 313, 314, 315, 317, 321, 326, 328, 331, 333, 335, 336, 337, 384, 387, 394, 425, 426, 428, 435, 440, 448, 457, 460, 466, 486, 499, 500, 504, 512, 514, 515, 516, 518, 524, 630, 668, 799, 800, 804, 812, 817, 858, 860, 861, 919, 947, 1007, 1008, 1059, 1072, 1129, 1130, 1161, 1272, 1275, 1287, 1403, 1404, 1484, 1529, 1572, 1579, 1580, 1582, 1583, 1584, 1587, 1588, 1589, 1590, 1591, 1593, 1594, 1595, 1596, 1606, 1607, 1609, 1632, 1697, 1745, 1753, 1754, 1763, 1779, 1798, 1815, 1820.

ALA1	ALANATE
ABA1	AMINOBUTYRATE
ARG0	ARGININE
ASN1	ASPARAGINATE
ASP2	ASPARTATE
CIT1	CITRULLINATE
CYS2	CYSTEINATE
CIS2	CYSTINATE
GLU2	GLUTAMATE
GLN1	GLUTAMINATE
GLY1	GLYCINATE
HIS1	HISTIDINATE
HYP1	HYDROXYPROLINATE
ILE1	ISOLEUCINATE
LEU1	LEUCINATE
LYS1	LYSINATE
MET1	METHIONATE
ORN1	ORNITHINATE
PHE1	PHENYLALANATE
PRO1	PROLINATE
SER1	SERINATE
THR1	THREONINATE
TRP1	TRYPTOPHANATE
TYR2	TYROSINATE
VAL1	VALINATE
HSN0	HISTAMINE
CO32	CARBONATE
PO43	PHOSPHATE
SIL2	SILICATE
SO42	SULPHATE
SCN1	THIOCYANATE
NH30	AMMONIA
ACA2	ASCORBATE
CTA3	CITRATE
LTA1	LACTATE
MLA2	MALATE
OXA2	OXALATE
PVA1	PYRUVATE
SLA2	SALICYLATE
SCA2	SUCCINATE
TRA2	TARTRATE
OPN2	OX-PENICILLAMINATE
GSH3	RED-GLUTATHIONATE
GSS4	OX-GLUTATHIONATE
PEN2	RED-PENICILLAMINATE
TET0	TRIETHYLTETRAMINE
EDT4	EDTA
GH 1	GLYCYLHISTIDINATE
GGH1	DIGLYCYLHISTIDINATE
PMA0	PEPTIDE MIMICKING ALBUMIN (N-METHYL-DIGLYCYLHISTIDINE)
BAL2	2,3 DIMERCAPTOPROPANOL (BAL)
DFO2	DESFERRIOXAMINE
DTP5	DTPA

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CDT4      CDTA
ACT1      ACETATE
ANT1      ANTHRANILATE
ASA1      ACETYLSALICYLIC ACID
CIQ1      CARBOXY-ISOQUINOLINE
FEN0      FENAMOLE
ATH1      BENZOYLATED THIAPROLINE D12267
SGL2      D12408
THP1      5,5-DIMETHYLTHIAZOLIDINE-4-CARBOXYLIC ACID
DPA1      2,3-DIAMINOPROPIONATE
EDD2      EDDA (ETHYLENEDIAMINEDIACETIC ACID).
NTA3      NTA
EGT4      EGTA
EHP4      EHPG
HDT3      HOEDTA
ODT4      EEDTA
H +1
OH-1
CA+2
CD+2
CO+2
CU+1
CU+2
FE+2
FE+3
PB+2
MG+2
MN+2
NI+2
ZN+2
CARB      PROTEIN SIMULATOR - CARBOXYLATE FUNCTIONS
TFRN      PROTEIN SIMULATOR - TRANSFERRIN
ALBM      PROTEIN SIMULATOR - ALBUMIN
END
13.187     ALBM(+1) CU+2(+1)                                    A
 8.187     ALBM(+1) PB+2(+1)                                    A
 6.187     ALBM(+1) MN+2(+1)                                    A
 7.187     ALBM(+1) ZN+2(+1)                                    A
22.528     TFRN(+1) FE+3(+1)                                    A
 1.142     CARB(+1) CA+2(+1)                                    A
 0.861     CARB(+1) MG+2(+1)                                    A
 9.502     ALA1(+1) H +1(+1)                                    M
11.879     ALA1(+1) H +1(+2)                                    M
 1.20      ALA1(+1) CA+2(+1)                                    A
10.10      ALA1(+1) CA+2(+1) H +1(+1)                           G
 1.80      ALA1(+2) CA+2(+1)                                    G
19.90      ALA1(+2) CA+2(+1) H +1(+2)                           G
 8.01      ALA1(+1) CU+2(+1)                                    M
14.64      ALA1(+2) CU+2(+1)                                    M
10.57      ALA1(+1) CU+2(+1) H +1(+1)                           M
 0.40      ALA1(+1) CU+2(+1) H +1(-1)                           E
 4.00      ALA1(+2) CU+2(+1) H +1(-1)                           E
 3.50      ALA1(+1) FE+2(+1)                                    A
 6.90      ALA1(+2) FE+2(+1)                                    A
10.00      ALA1(+1) FE+3(+1)                                    A
```

16.40	ALA1(+2)	FE+3(+1)	G
4.20	ALA1(+1)	PB+2(+1)	A
6.90	ALA1(+2)	PB+2(+1)	A
9.10	ALA1(+2)	PB+2(+1) OH-1(+1)	E
1.70	ALA1(+1)	MG+2(+1)	A
10.40	ALA1(+1)	MG+2(+1) H +1(+1)	G
2.20	ALA1(+2)	MG+2(+1)	G
20.20	ALA1(+2)	MG+2(+1) H +1(+2)	G
2.40	ALA1(+1)	MN+2(+1)	M
4.28	ALA1(+2)	MN+2(+1)	M
5.70	ALA1(+3)	MN+2(+1)	M
10.00	ALA1(+1)	MN+2(+1) H +1(+1)	G
12.85	ALA1(+2)	MN+2(+1) H +1(+1)	M
5.40	ALA1(+1)	NI+2(+1)	A
9.60	ALA1(+2)	NI+2(+1)	A
4.57	ALA1(+1)	ZN+2(+1)	M
8.56	ALA1(+2)	ZN+2(+1)	M
10.65	ALA1(+3)	ZN+2(+1)	M
-3.96	ALA1(+1)	ZN+2(+1) H +1(-1)	M
9.24	ABA1(+1)	H +1(+1)	M
11.69	ABA1(+1)	H +1(+2)	A
1.10	ABA1(+1)	CA+2(+1)	G
9.70	ABA1(+1)	CA+2(+1) H +1(+1)	G
1.40	ABA1(+2)	CA+2(+1)	G
19.20	ABA1(+2)	CA+2(+1) H +1(+2)	G
7.65	ABA1(+1)	CU+2(+1)	M
14.10	ABA1(+2)	CU+2(+1)	M
3.40	ABA1(+1)	FE+2(+1)	E
5.00	ABA1(+2)	FE+2(+1)	G
9.00	ABA1(+1)	FE+3(+1)	E
16.00	ABA1(+2)	FE+3(+1)	G
4.00	ABA1(+1)	PB+2(+1)	G
6.00	ABA1(+2)	PB+2(+1)	G
8.00	ABA1(+1)	PB+2(+1) OH-1(+1)	G
1.60	ABA1(+1)	MG+2(+1)	G
10.00	ABA1(+1)	MG+2(+1) H +1(+1)	G
2.20	ABA1(+2)	MG+2(+1)	G
19.50	ABA1(+2)	MG+2(+1) H +1(+2)	G
2.25	ABA1(+1)	MN+2(+1)	G
4.00	ABA1(+2)	MN+2(+1)	G
5.20	ABA1(+1)	NI+2(+1)	E
9.40	ABA1(+2)	NI+2(+1)	E
4.42	ABA1(+1)	ZN+2(+1)	M
8.15	ABA1(+2)	ZN+2(+1)	M
8.78	ARG0(+1)	H +1(+1)	M
10.81	ARG0(+1)	H +1(+2)	M
-12.50	ARG0(+1)	H +1(-1)	A
1.60	ARG0(+1)	CA+2(+1)	A
9.70	ARG0(+1)	CA+2(+1) H +1(+1)	G
2.20	ARG0(+2)	CA+2(+1)	G
18.80	ARG0(+2)	CA+2(+1) H +1(+2)	G
7.38	ARG0(+1)	CU+2(+1)	M
13.66	ARG0(+2)	CU+2(+1)	M
-1.00	ARG0(+1)	CU+2(+1) H +1(-1)	M
3.17	ARG0(+2)	CU+2(+2) H +1(-2)	M

3.00	ARG0(+1)	FE+2(+1)		A
4.40	ARG0(+2)	FE+2(+1)		G
8.00	ARG0(+1)	FE+3(+1)		A
15.00	ARG0(+2)	FE+3(+1)		G
3.50	ARG0(+1)	PB+2(+1)		A
5.00	ARG0(+2)	PB+2(+1)		G
8.00	ARG0(+1)	PB+2(+1)	OH-1(+1)	G
2.00	ARG0(+1)	MG+2(+1)		A
10.30	ARG0(+1)	MG+2(+1)	H +1(+1)	G
2.30	ARG0(+2)	MG+2(+1)		G
19.00	ARG0(+2)	MG+2(+1)	H +1(+2)	G
2.35	ARG0(+1)	MN+2(+1)		A
3.90	ARG0(+2)	MN+2(+1)		A
5.00	ARG0(+1)	NI+2(+1)		A
9.00	ARG0(+2)	NI+2(+1)		A
4.07	ARG0(+1)	ZN+2(+1)		M
7.88	ARG0(+2)	ZN+2(+1)		M
-4.25	ARG0(+1)	ZN+2(+1)	H +1(-1)	M
8.68	ASN1(+1)	H +1(+1)		M
10.91	ASN1(+1)	H +1(+2)		M
1.40	ASN1(+1)	CA+2(+1)		G
9.40	ASN1(+1)	CA+2(+1)	H +1(+1)	G
1.70	ASN1(+2)	CA+2(+1)		G
13.40	ASN1(+2)	CA+2(+1)	H +1(+2)	G
7.69	ASN1(+1)	CU+2(+1)		M
13.66	ASN1(+2)	CU+2(+1)		M
3.50	ASN1(+2)	CU+2(+1)	H +1(-1)	E
3.40	ASN1(+1)	FE+2(+1)		A
6.00	ASN1(+2)	FE+2(+1)		A
8.00	ASN1(+3)	FE+2(+1)		E
8.40	ASN1(+1)	FE+3(+1)		A
14.75	ASN1(+2)	FE+3(+1)		G
4.00	ASN1(+1)	PB+2(+1)		A
6.00	ASN1(+2)	PB+2(+1)		A
7.00	ASN1(+3)	PB+2(+1)		E
9.20	ASN1(+2)	PB+2(+1)	OH-1(+1)	E
1.80	ASN1(+1)	MG+2(+1)		G
9.70	ASN1(+1)	MG+2(+1)	H +1(+1)	G
2.30	ASN1(+2)	MG+2(+1)		E
18.70	ASN1(+2)	MG+2(+1)	H +1(+2)	G
2.40	ASN1(+1)	MN+2(+1)		E
4.00	ASN1(+2)	MN+2(+1)		E
5.30	ASN1(+1)	NI+2(+1)		A
9.40	ASN1(+2)	NI+2(+1)		A
11.40	ASN1(+3)	NI+2(+1)		G
4.45	ASN1(+1)	ZN+2(+1)		M
7.95	ASN1(+2)	ZN+2(+1)		M
10.00	ASN1(+3)	ZN+2(+1)		E
9.266	ASP2(+1)	H +1(+1)		M
12.86	ASP2(+1)	H +1(+2)		M
14.81	ASP2(+1)	H +1(+3)		M
1.60	ASP2(+1)	CA+2(+1)		A
10.20	ASP2(+1)	CA+2(+1)	H +1(+1)	G
2.10	ASP2(+2)	CA+2(+1)		G
19.70	ASP2(+2)	CA+2(+1)	H +1(+2)	G

8.50	ASP2(+1) CU+2(+1)		A
15.20	ASP2(+2) CU+2(+1)		A
12.20	ASP2(+1) CU+2(+1)	H +1(+1)	E
20.60	ASP2(+2) CU+2(+1)	H +1(+1)	E
24.00	ASP2(+2) CU+2(+1)	H +1(+2)	G
4.20	ASP2(+1) FE+2(+1)		A
7.75	ASP2(+2) FE+2(+1)		G
11.00	ASP2(+1) FE+3(+1)		A
17.10	ASP2(+2) FE+3(+1)		G
5.80	ASP2(+1) PB+2(+1)		A
8.20	ASP2(+2) PB+2(+1)		A
10.70	ASP2(+1) PB+2(+1)	H +1(+1)	E
-4.00	ASP2(+1) PB+2(+1)	H +1(-1)	E
2.20	ASP2(+1) MG+2(+1)		A
10.60	ASP2(+1) MG+2(+1)	H +1(+1)	G
2.90	ASP2(+2) MG+2(+1)		G
20.10	ASP2(+2) MG+2(+1)	H +1(+2)	G
3.20	ASP2(+1) MN+2(+1)		A
5.20	ASP2(+2) MN+2(+1)		G
6.80	ASP2(+1) NI+2(+1)		A
10.50	ASP2(+1) NI+2(+1)	H +1(+1)	G
11.60	ASP2(+2) NI+2(+1)		A
6.01	ASP2(+1) ZN+2(+1)		E
11.88	ASP2(+1) ZN+2(+1)	H +1(+1)	G
9.30	ASP2(+2) ZN+2(+1)		A
8.70	CIT1(+1) H +1(+1)		A
10.40	CIT1(+1) H +1(+2)		A
1.20	CIT1(+1) CA+2(+1)		A
9.30	CIT1(+1) CA+2(+1)	H +1(+1)	G
1.50	CIT1(+2) CA+2(+1)		A
18.30	CIT1(+2) CA+2(+1)	H +1(+2)	G
7.00	CIT1(+1) CU+2(+1)		A
13.00	CIT1(+2) CU+2(+1)		A
3.00	CIT1(+1) FE+2(+1)		G
4.80	CIT1(+2) FE+2(+1)		G
8.00	CIT1(+1) FE+3(+1)		G
14.42	CIT1(+2) FE+3(+1)		G
4.00	CIT1(+1) PB+2(+1)		E
6.00	CIT1(+2) PB+2(+1)		G
8.00	CIT1(+1) PB+2(+1)	OH-1(+1)	G
1.60	CIT1(+1) MG+2(+1)		A
9.80	CIT1(+1) MG+2(+1)	H +1(+1)	G
1.90	CIT1(+2) MG+2(+1)		A
18.80	CIT1(+2) MG+2(+1)	H +1(+2)	G
1.70	CIT1(+1) MN+2(+1)		A
2.60	CIT1(+2) MN+2(+1)		G
4.75	CIT1(+1) NI+2(+1)		E
8.30	CIT1(+2) NI+2(+1)		E
4.00	CIT1(+1) ZN+2(+1)		A
7.00	CIT1(+2) ZN+2(+1)		A
10.113	CYS2(+1) H +1(+1)		M
18.04	CYS2(+1) H +1(+2)		M
20.00	CYS2(+1) H +1(+3)		M
2.30	CYS2(+1) CA+2(+1)		A
11.20	CYS2(+1) CA+2(+1)	H +1(+1)	G

2.70	CYS2(+2)	CA+2(+1)	G
21.70	CYS2(+2)	CA+2(+1) H +1(+2)	G
13.00	CYS2(+1)	CU+1(+1)	G
17.00	CYS2(+2)	CU+1(+1)	G
20.50	CYS2(+1)	CU+1(+1) H +1(+1)	G
35.50	CYS2(+2)	CU+1(+1) H +1(+2)	G
94.00	CYS2(+4)	CU+1(+5)	G
6.00	CYS2(+1)	FE+2(+1)	A
11.00	CYS2(+2)	FE+2(+1)	A
11.73	CYS2(+1)	PB+2(+1)	M
15.48	CYS2(+1)	PB+2(+1) H +1(+1)	M
16.00	CYS2(+2)	PB+2(+1)	A
18.40	CYS2(+3)	PB+2(+1)	A
26.00	CYS2(+2)	PB+2(+1) H +1(+1)	E
31.00	CYS2(+2)	PB+2(+1) H +1(+2)	G
2.60	CYS2(+1)	MG+2(+1)	E
11.50	CYS2(+1)	MG+2(+1) H +1(+1)	G
3.40	CYS2(+2)	MG+2(+1)	G
22.00	CYS2(+2)	MG+2(+1) H +1(+2)	G
4.10	CYS2(+1)	MN+2(+1)	A
7.10	CYS2(+2)	MN+2(+1)	A
9.61	CYS2(+1)	NI+2(+1)	M
19.23	CYS2(+2)	NI+2(+1)	M
8.60	CYS2(+1)	ZN+2(+1)	M
17.77	CYS2(+2)	ZN+2(+1)	M
14.60	CYS2(+1)	ZN+2(+1) H +1(+1)	M
24.11	CYS2(+2)	ZN+2(+1) H +1(+1)	M
29.72	CYS2(+2)	ZN+2(+1) H +1(+2)	M
20.50	CYS2(+3)	ZN+2(+1)	M
30.16	CYS2(+3)	ZN+2(+2)	M
36.39	CYS2(+3)	ZN+2(+2) H +1(+1)	M
41.75	CYS2(+3)	ZN+2(+2) H +1(+2)	M
42.68	CYS2(+4)	ZN+2(+3)	M
48.21	CYS2(+4)	ZN+2(+3) H +1(+1)	M
53.52	CYS2(+4)	ZN+2(+3) H +1(+2)	M
8.69	CIS2(+1)	H +1(+1)	M
16.64	CIS2(+1)	H +1(+2)	M
18.34	CIS2(+1)	H +1(+3)	M
2.0	CIS2(+1)	CA+2(+1)	G
2.5	CIS2(+2)	CA+2(+1)	G
10.00	CIS2(+1)	CA+2(+1) H +1(+1)	G
17.40	CIS2(+1)	CA+2(+1) H +1(+2)	G
19.50	CIS2(+2)	CA+2(+1) H +1(+2)	G
34.20	CIS2(+2)	CA+2(+1) H +1(+4)	G
6.80	CIS2(+1)	CU+2(+1)	G
16.87	CIS2(+1)	CU+2(+1) H +1(+1)	M
16.08	CIS2(+1)	CU+2(+2)	M
30.18	CIS2(+2)	CU+2(+2)	M
29.00	CIS2(+2)	CU+2(+1) H +1(+2)	E
17.70	CIS2(+1)	FE+3(+1) H +1(+1)	G
25.80	CIS2(+2)	FE+3(+1) H +1(+1)	G
31.28	CIS2(+2)	FE+3(+1) H +1(+2)	G
16.20	CIS2(+1)	PB+2(+1) H +1(+1)	G
22.00	CIS2(+2)	PB+2(+1) H +1(+2)	G
10.70	CIS2(+1)	MG+2(+1) H +1(+1)	G

17.70	CIS2(+1)	MG+2(+1)	H +1(+2)	G
20.00	CIS2(+2)	MG+2(+1)	H +1(+2)	G
34.50	CIS2(+2)	MG+2(+1)	H +1(+4)	G
11.00	CIS2(+1)	MN+2(+1)	H +1(+1)	G
23.00	CIS2(+2)	MN+2(+1)	H +1(+2)	G
5.50	CIS2(+1)	NI+2(+1)		G
14.40	CIS2(+1)	NI+2(+1)	H +1(+1)	G
27.20	CIS2(+2)	NI+2(+1)	H +1(+2)	G
13.33	CIS2(+1)	ZN+2(+1)	H +1(+1)	M
26.00	CIS2(+2)	ZN+2(+1)	H +1(+2)	M
9.39	GLU2(+1)	H +1(+1)		M
13.54	GLU2(+1)	H +1(+2)		M
15.67	GLU2(+1)	H +1(+3)		M
1.50	GLU2(+1)	CA+2(+1)		A
10.40	GLU2(+1)	CA+2(+1)	H +1(+1)	A
1.90	GLU2(+2)	CA+2(+1)		A
20.10	GLU2(+2)	CA+2(+1)	H +1(+2)	A
8.74	GLU2(+1)	CU+2(+1)		M
14.91	GLU2(+2)	CU+2(+1)		M
12.79	GLU2(+1)	CU+2(+1)	H +1(+1)	M
20.00	GLU2(+2)	CU+2(+1)	H +1(+1)	G
24.50	GLU2(+2)	CU+2(+1)	H +1(+2)	G
3.40	GLU2(+1)	FE+2(+1)		A
6.00	GLU2(+2)	FE+2(+1)		G
11.90	GLU2(+1)	FE+3(+1)		E
18.70	GLU2(+2)	FE+3(+1)		G
4.50	GLU2(+1)	PB+2(+1)		E
6.00	GLU2(+2)	PB+2(+1)		E
8.30	GLU2(+1)	PB+2(+1)	OH-1(+1)	G
9.70	GLU2(+2)	PB+2(+1)	OH-1(+1)	G
1.80	GLU2(+1)	MG+2(+1)		A
10.60	GLU2(+1)	MG+2(+1)	H +1(+1)	G
2.50	GLU2(+2)	MG+2(+1)		G
20.30	GLU2(+2)	MG+2(+1)	H +1(+2)	G
3.05	GLU2(+1)	MN+2(+1)		M
4.90	GLU2(+2)	MN+2(+1)		G
5.40	GLU2(+1)	NI+2(+1)		A
9.30	GLU2(+2)	NI+2(+1)		A
11.30	GLU2(+3)	NI+2(+1)		G
4.76	GLU2(+1)	ZN+2(+1)		M
8.54	GLU2(+2)	ZN+2(+1)		M
9.80	GLU2(+3)	ZN+2(+1)		A
8.68	GLN1(+1)	H +1(+1)		M
10.86	GLN1(+1)	H +1(+2)		M
1.00	GLN1(+1)	CA+2(+1)		G
9.40	GLN1(+1)	CA+2(+1)	H +1(+1)	G
1.40	GLN1(+2)	CA+2(+1)		G
18.40	GLN1(+2)	CA+2(+1)	H +1(+2)	G
7.48	GLN1(+1)	CU+2(+1)		M
13.57	GLN1(+2)	CU+2(+1)		M
3.50	GLN1(+1)	FE+2(+1)		A
6.00	GLN1(+2)	FE+2(+1)		A
8.00	GLN1(+3)	FE+2(+1)		A
8.00	GLN1(+1)	FE+3(+1)		G
14.79	GLN1(+2)	FE+3(+1)		

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 4.00      GLN1(+1)  PB+2(+1)                          E
 7.00      GLN1(+2)  PB+2(+1)                          E
 8.00      GLN1(+3)  PB+2(+1)                          E
 8.00      GLN1(+1)  PB+2(+1)  OH-1(+1)                G
 1.70      GLN1(+1)  MG+2(+1)                          G
 9.80      GLN1(+1)  MG+2(+1)  H +1(+1)                G
 2.20      GLN1(+2)  MG+2(+1)                          G
18.90      GLN1(+2)  MG+2(+1)  H +1(+2)                G
 2.60      GLN1(+1)  MN+2(+1)                          A
 4.00      GLN1(+2)  MN+2(+1)                          A
 4.90      GLN1(+1)  NI+2(+1)                          A
 8.90      GLN1(+2)  NI+2(+1)                          A
11.10      GLN1(+3)  NI+2(+1)                          G
 4.17      GLN1(+1)  ZN+2(+1)                          M
 7.66      GLN1(+2)  ZN+2(+1)                          M
10.00      GLN1(+3)  ZN+2(+1)                          E
 9.270     GLY1(+1)  H +1(+1)                          M
11.625     GLY1(+1)  H +1(+2)                          M
 1.30      GLY1(+1)  CA+2(+1)                          A
10.10      GLY1(+1)  CA+2(+1)  H +1(+1)                G
 1.70      GLY1(+2)  CA+2(+1)                          G
19.80      GLY1(+2)  CA+2(+1)  H +1(+2)                G
 8.02      GLY1(+1)  CU+2(+1)                          M
14.67      GLY1(+2)  CU+2(+1)                          M
10.11      GLY1(+1)  CU+2(+1)  H +1(+1)                M
 3.50      GLY1(+1)  FE+2(+1)                          A
 5.50      GLY1(+2)  FE+2(+1)                          A
 9.40      GLY1(+1)  FE+3(+1)                          A
16.50      GLY1(+2)  FE+3(+1)                          G
 5.00      GLY1(+1)  PB+2(+1)                          A
 8.50      GLY1(+2)  PB+2(+1)                          A
12.20      GLY1(+1)  PB+2(+1)  H +1(+1)                E
-3.00      GLY1(+1)  PB+2(+1)  H +1(-1)                A
 2.10      GLY1(+1)  MG+2(+1)                          A
10.60      GLY1(+1)  MG+2(+1)  H +1(+1)                G
 2.70      GLY1(+2)  MG+2(+1)                          G
20.30      GLY1(+2)  MG+2(+1)  H +1(+2)                G
 2.71      GLY1(+1)  MN+2(+1)                          M
 4.76      GLY1(+2)  MN+2(+1)                          M
 5.52      GLY1(+3)  MN+2(+1)                          M
10.02      GLY1(+1)  MN+2(+1)  H +1(+1)                M
12.89      GLY1(+2)  MN+2(+1)  H +1(+1)                M
 5.70      GLY1(+1)  NI+2(+1)                          M
10.30      GLY1(+2)  NI+2(+1)                          M
13.50      GLY1(+3)  NI+2(+1)                          M
 4.83      GLY1(+1)  ZN+2(+1)                          M
 8.93      GLY1(+2)  ZN+2(+1)                          M
10.77      GLY1(+3)  ZN+2(+1)                          M
10.07      GLY1(+1)  ZN+2(+1)  H +1(+1)                M
-3.70      GLY1(+1)  ZN+2(+1)  H +1(-1)                M
 8.777     HIS1(+1)  H +1(+1)                          M
14.60      HIS1(+1)  H +1(+2)                          M
16.29      HIS1(+1)  H +1(+3)                          M
 1.40      HIS1(+1)  CA+2(+1)                          G
 9.70      HIS1(+1)  CA+2(+1)  H +1(+1)                G
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1.80	HIS1(+2) CA+2(+1)	G
18.90	HIS1(+2) CA+2(+1) H +1(+2)	G
9.75	HIS1(+1) CU+2(+1)	M
17.40	HIS1(+2) CU+2(+1)	M
13.70	HIS1(+1) CU+2(+1) H +1(+1)	M
2.39	HIS1(+1) CU+2(+1) H +1(-1)	M
22.96	HIS1(+2) CU+2(+1) H +1(+1)	M
7.50	HIS1(+2) CU+2(+2) H +1(-2)	M
26.16	HIS1(+2) CU+2(+1) H +1(+2)	M
5.20	HIS1(+1) FE+2(+1)	A
9.50	HIS1(+2) FE+2(+1)	A
4.20	HIS1(+1) FE+3(+1)	G
7.65	HIS1(+2) FE+3(+1)	G
5.96	HIS1(+1) PB+2(+1)	M
9.50	HIS1(+2) PB+2(+1)	M
16.80	HIS1(+2) PB+2(+1) H +1(+1)	E
22.50	HIS1(+2) PB+2(+1) H +1(+2)	E
8.00	HIS1(+1) PB+2(+1) OH-1(+1)	G
2.20	HIS1(+1) MG+2(+1)	G
10.10	HIS1(+1) MG+2(+1) H +1(+1)	G
2.90	HIS1(+2) MG+2(+1)	G
19.30	HIS1(+2) MG+2(+1) H +1(+2)	G
3.24	HIS1(+1) MN+2(+1)	M
6.16	HIS1(+2) MN+2(+1)	M
8.32	HIS1(+1) NI+2(+1)	M
14.86	HIS1(+2) NI+2(+1)	M
10.25	HIS1(+1) NI+2(+1) H +1(+1)	M
18.00	HIS1(+2) NI+2(+1) H +1(+1)	A
6.29	HIS1(+1) ZN+2(+1)	M
11.43	HIS1(+2) ZN+2(+1)	M
10.50	HIS1(+1) ZN+2(+1) H +1(+1)	M
16.00	HIS1(+2) ZN+2(+1) H +1(+1)	M
21.00	HIS1(+2) ZN+2(+1) H +1(+2)	E
9.426	HSNO(+1) H +1(+1)	M
15.32	HSNO(+1) H +1(+2)	M
9.16	HSNO(+1) CU+2(+1)	M
15.48	HSNO(+2) CU+2(+1)	M
12.58	HSNO(+1) CU+2(+1) H +1(+1)	M
-2.00	HSNO(+1) CU+2(+1) H +1(-1)	G
4.22	HSNO(+2) CU+2(+1) H +1(-1)	M
21.02	HSNO(+2) CU+2(+1) H +1(+1)	M
7.06	HSNO(+2) CU+2(+2) H +1(-2)	M
-5.95	HSNO(+2) CU+2(+1) H +1(-2)	M
4.55	HSNO(+1) CD+2(+1)	M
7.73	HSNO(+2) CD+2(+1)	M
-5.39	HSNO(+1) CD+2(+1) H +1(-1)	M
4.90	HSNO(+1) CO+2(+1)	M
8.63	HSNO(+2) CO+2(+1)	M
10.67	HSNO(+3) CO+2(+1)	M
-1.48	HSNO(+2) CO+2(+1) H +1(-1)	M
4.40	HSNO(+1) FE+2(+1)	G
8.00	HSNO(+2) FE+2(+1)	G
5.00	HSNO(+1) FE+3(+1)	G
9.00	HSNO(+2) FE+3(+1)	G
2.00	HSNO(+1) MN+2(+1)	G

3.40	HSNO(+2)	MN+2(+1)	G
6.46	HSNO(+1)	NI+2(+1)	M
11.31	HSNO(+2)	NI+2(+1)	M
14.28	HSNO(+3)	NI+2(+1)	M
11.48	HSNO(+1)	NI+2(+1) H +1(+1)	M
-2.96	HSNO(+1)	NI+2(+1) H +1(-1)	M
0.00	HSNO(+2)	NI+2(+1) H +1(-1)	G
2.78	HSNO(+3)	NI+2(+1) H +1(-1)	M
4.87	HSNO(+1)	ZN+2(+1)	M
9.65	HSNO(+2)	ZN+2(+1)	M
11.50	HSNO(+3)	ZN+2(+1)	M
-2.74	HSNO(+1)	ZN+2(+1) H +1(-1)	M
9.30	HYP1(+1)	H +1(+1)	A
11.20	HYP1(+1)	H +1(+2)	A
1.00	HYP1(+1)	CA+2(+1)	A
9.80	HYP1(+1)	CA+2(+1) H +1(+1)	G
1.30	HYP1(+2)	CA+2(+1)	G
19.40	HYP1(+2)	CA+2(+1) H +1(+2)	G
8.10	HYP1(+1)	CU+2(+1)	A
14.50	HYP1(+2)	CU+2(+1)	A
3.80	HYP1(+1)	FE+2(+1)	A
6.00	HYP1(+2)	FE+2(+1)	G
8.50	HYP1(+1)	FE+3(+1)	G
15.00	HYP1(+2)	FE+3(+1)	G
3.50	HYP1(+1)	PB+2(+1)	G
4.80	HYP1(+2)	PB+2(+1)	G
8.00	HYP1(+1)	PB+2(+1) OH-1(+1)	G
1.70	HYP1(+1)	MG+2(+1)	G
10.20	HYP1(+1)	MG+2(+1) H +1(+1)	G
2.30	HYP1(+2)	MG+2(+1)	G
19.80	HYP1(+2)	MG+2(+1) H +1(+2)	G
2.70	HYP1(+1)	MN+2(+1)	G
4.90	HYP1(+2)	MN+2(+1)	G
5.70	HYP1(+1)	NI+2(+1)	A
10.00	HYP1(+2)	NI+2(+1)	A
4.50	HYP1(+1)	ZN+2(+1)	A
8.50	HYP1(+2)	ZN+2(+1)	A
9.36	ILE1(+1)	H +1(+1)	M
11.72	ILE1(+1)	H +1(+2)	M
1.10	ILE1(+1)	CA+2(+1)	A
9.90	ILE1(+1)	CA+2(+1) H +1(+1)	G
1.40	ILE1(+2)	CA+2(+1)	G
19.60	ILE1(+2)	CA+2(+1) H +1(+2)	G
7.95	ILE1(+1)	CU+2(+1)	M
14.68	ILE1(+2)	CU+2(+1)	M
0.32	ILE1(+1)	CU+2(+1) H +1(-1)	M
3.00	ILE1(+1)	FE+2(+1)	G
5.00	ILE1(+2)	FE+2(+1)	G
9.00	ILE1(+1)	FE+3(+1)	G
15.40	ILE1(+2)	FE+3(+1)	G
3.80	ILE1(+1)	PB+2(+1)	E
5.30	ILE1(+2)	PB+2(+1)	G
8.00	ILE1(+1)	PB+2(+1) OH-1(+1)	E
1.80	ILE1(+1)	MG+2(+1)	G
10.30	ILE1(+1)	MG+2(+1) H +1(+1)	G

2.40	ILE1(+2)	MG+2(+1)	G
20.10	ILE1(+2)	MG+2(+1) H +1(+2)	G
2.60	ILE1(+1)	MN+2(+1)	A
4.70	ILE1(+2)	MN+2(+1)	A
5.20	ILE1(+1)	NI+2(+1)	A
9.40	ILE1(+2)	NI+2(+1)	A
11.00	ILE1(+3)	NI+2(+1)	A
4.40	ILE1(+1)	ZN+2(+1)	M
8.08	ILE1(+2)	ZN+2(+1)	M
10.00	ILE1(+3)	ZN+2(+1)	A
-3.62	ILE1(+1)	ZN+2(+1) H +1(-1)	M
15.25	ILE1(+2)	ZN+2(+1) H +1(+1)	M
9.36	LEU1(+1)	H +1(+1)	M
11.72	LEU1(+1)	H +1(+2)	M
1.10	LEU1(+1)	CA+2(+1)	A
9.90	LEU1(+1)	CA+2(+1) H +1(+1)	G
1.40	LEU1(+2)	CA+2(+1)	G
19.60	LEU1(+2)	CA+2(+1) H +1(+2)	G
8.04	LEU1(+1)	CU+2(+1)	M
14.69	LEU1(+2)	CU+2(+1)	M
11.49	LEU1(+1)	CU+2(+1) H +1(+1)	M
19.43	LEU1(+2)	CU+2(+1) H +1(+1)	M
3.32	LEU1(+1)	FE+2(+1)	A
5.00	LEU1(+2)	FE+2(+1)	E
9.49	LEU1(+1)	FE+3(+1)	A
15.50	LEU1(+2)	FE+3(+1)	E
4.00	LEU1(+1)	PB+2(+1)	G
5.50	LEU1(+2)	PB+2(+1)	G
8.00	LEU1(+1)	PB+2(+1) OH-1(+1)	G
1.80	LEU1(+1)	MG+2(+1)	G
10.40	LEU1(+1)	MG+2(+1) H +1(+1)	G
2.30	LEU1(+2)	MG+2(+1)	G
20.10	LEU1(+2)	MG+2(+1) H +1(+2)	G
2.30	LEU1(+1)	MN+2(+1)	A
4.20	LEU1(+2)	MN+2(+1)	A
5.40	LEU1(+1)	NI+2(+1)	A
9.40	LEU1(+2)	NI+2(+1)	A
12.90	LEU1(+3)	NI+2(+1)	A
4.51	LEU1(+1)	ZN+2(+1)	M
8.56	LEU1(+2)	ZN+2(+1)	M
10.60	LEU1(+3)	ZN+2(+1)	G
-4.25	LEU1(+1)	ZN+2(+1) H +1(-1)	G
15.17	LEU1(+2)	ZN+2(+1) H +1(+1)	M
10.30	LYS1(+1)	H +1(+1)	M
19.18	LYS1(+1)	H +1(+2)	M
21.33	LYS1(+1)	H +1(+3)	M
1.0	LYS1(+1)	CA+2(+1)	G
1.3	LYS1(+2)	CA+2(+1)	G
11.60	LYS1(+1)	CA+2(+1) H +1(+1)	G
20.75	LYS1(+1)	CA+2(+1) H +1(+2)	G
21.80	LYS1(+2)	CA+2(+1) H +1(+2)	G
39.70	LYS1(+2)	CA+2(+1) H +1(+4)	G
10.85	LYS1(+1)	CU+2(+1)	M
14.60	LYS1(+2)	CU+2(+1)	M
17.99	LYS1(+1)	CU+2(+1) H +1(+1)	M

20.64	LYS1(+1)	CU+2(+1)	H +1(+2)	M
25.62	LYS1(+2)	CU+2(+1)	H +1(+1)	M
34.80	LYS1(+2)	CU+2(+1)	H +1(+2)	M
13.80	LYS1(+1)	FE+2(+1)	H +1(+1)	A
18.30	LYS1(+2)	FE+2(+1)	H +1(+1)	G
25.00	LYS1(+2)	FE+2(+1)	H +1(+2)	G
18.40	LYS1(+1)	FE+3(+1)	H +1(+1)	G
28.88	LYS1(+2)	FE+3(+1)	H +1(+1)	G
35.87	LYS1(+2)	FE+3(+1)	H +1(+2)	G
14.10	LYS1(+1)	PB+2(+1)	H +1(+1)	G
18.90	LYS1(+2)	PB+2(+1)	H +1(+1)	G
25.86	LYS1(+2)	PB+2(+1)	H +1(+2)	G
1.10	LYS1(+1)	MG+2(+1)		G
1.40	LYS1(+2)	MG+2(+1)		G
12.10	LYS1(+1)	MG+2(+1)	H +1(+1)	G
21.20	LYS1(+1)	MG+2(+1)	H +1(+2)	G
22.70	LYS1(+2)	MG+2(+1)	H +1(+2)	G
40.00	LYS1(+2)	MG+2(+1)	H +1(+4)	G
12.20	LYS1(+1)	MN+2(+1)	H +1(+1)	A
19.50	LYS1(+2)	MN+2(+1)	H +1(+1)	G
24.35	LYS1(+2)	MN+2(+1)	H +1(+2)	G
5.50	LYS1(+1)	NI+2(+1)		G
9.00	LYS1(+2)	NI+2(+1)		G
16.00	LYS1(+1)	NI+2(+1)	H +1(+1)	A
21.50	LYS1(+2)	NI+2(+1)	H +1(+1)	G
29.80	LYS1(+2)	NI+2(+1)	H +1(+2)	A
3.50	LYS1(+1)	ZN+2(+1)		G
7.00	LYS1(+2)	ZN+2(+1)		G
14.37	LYS1(+1)	ZN+2(+1)	H +1(+1)	M
19.84	LYS1(+2)	ZN+2(+1)	H +1(+1)	M
28.51	LYS1(+2)	ZN+2(+1)	H +1(+2)	M
8.91	MET1(+1)	H +1(+1)		M
11.17	MET1(+1)	H +1(+2)		M
1.20	MET1(+1)	CA+2(+1)		A
9.50	MET1(+1)	CA+2(+1)	H +1(+1)	G
1.60	MET1(+2)	CA+2(+1)		G
18.70	MET1(+2)	CA+2(+1)	H +1(+2)	G
7.67	MET1(+1)	CU+2(+1)		M
14.08	MET1(+2)	CU+2(+1)		M
3.15	MET1(+1)	FE+2(+1)		A
4.90	MET1(+2)	FE+2(+1)		G
8.60	MET1(+1)	FE+3(+1)		A
14.94	MET1(+2)	FE+3(+1)		G
4.30	MET1(+1)	PB+2(+1)		A
7.50	MET1(+2)	PB+2(+1)		E
8.20	MET1(+1)	PB+2(+1)	OH-1(+1)	G
1.80	MET1(+1)	MG+2(+1)		G
9.90	MET1(+1)	MG+2(+1)	H +1(+1)	G
2.40	MET1(+2)	MG+2(+1)		G
19.10	MET1(+2)	MG+2(+1)	H +1(+2)	G
2.70	MET1(+1)	MN+2(+1)		A
4.25	MET1(+2)	MN+2(+1)		A
5.00	MET1(+1)	NI+2(+1)		A
9.10	MET1(+2)	NI+2(+1)		A
11.10	MET1(+3)	NI+2(+1)		A

4.22	MET1(+1) ZN+2(+1)	M
6.93	MET1(+2) ZN+2(+1)	M
10.22	ORN1(+1) H +1(+1)	M
18.77	ORN1(+1) H +1(+2)	M
20.69	ORN1(+1) H +1(+3)	M
1.0	ORN1(+1) CA+2(+1)	G
1.30	ORN1(+2) CA+2(+1)	G
19.40	ORN1(+1) CA+2(+1) H +1(+2)	G
11.40	ORN1(+1) CA+2(+1) H +1(+1)	A
21.30	ORN1(+2) CA+2(+1) H +1(+2)	G
38.50	ORN1(+2) CA+2(+1) H +1(+4)	G
9.78	ORN1(+1) CU+2(+1)	M
14.77	ORN1(+2) CU+2(+1)	M
17.42	ORN1(+1) CU+2(+1) H +1(+1)	M
24.73	ORN1(+2) CU+2(+1) H +1(+1)	M
33.64	ORN1(+2) CU+2(+1) H +1(+2)	M
0.89	ORN1(+1) CU+2(+1) H +1(-1)	M
12.76	ORN1(+1) FE+2(+1) H +1(+1)	A
24.25	ORN1(+2) FE+2(+1) H +1(+2)	E
15.47	ORN1(+2) FE+2(+1) H +1(+1)	G
13.28	ORN1(+1) FE+3(+1) H +1(+1)	A
35.34	ORN1(+2) FE+3(+1) H +1(+2)	E
28.52	ORN1(+2) FE+3(+1) H +1(+1)	G
13.90	ORN1(+1) PB+2(+1) H +1(+1)	G
18.50	ORN1(+2) PB+2(+1) H +1(+1)	G
25.50	ORN1(+2) PB+2(+1) H +1(+2)	G
9.00	ORN1(+1) PB+2(+1) OH-1(+1)	G
11.90	ORN1(+1) MG+2(+1) H +1(+1)	A
19.70	ORN1(+1) MG+2(+1) H +1(+2)	G
22.20	ORN1(+2) MG+2(+1) H +1(+2)	G
38.80	ORN1(+2) MG+2(+1) H +1(+4)	G
12.02	ORN1(+1) MN+2(+1) H +1(+1)	G
19.00	ORN1(+2) MN+2(+1) H +1(+1)	G
23.70	ORN1(+2) MN+2(+1) H +1(+2)	G
7.00	ORN1(+1) NI+2(+1)	G
14.80	ORN1(+1) NI+2(+1) H +1(+1)	A
28.50	ORN1(+2) NI+2(+1) H +1(+2)	A
5.96	ORN1(+1) ZN+2(+1)	M
14.08	ORN1(+1) ZN+2(+1) H +1(+1)	M
19.31	ORN1(+2) ZN+2(+1) H +1(+1)	M
27.62	ORN1(+2) ZN+2(+1) H +1(+2)	M
-2.59	ORN1(+1) ZN+2(+1) H +1(-1)	M
8.78	PHE1(+1) H +1(+1)	M
10.97	PHE1(+1) H +1(+2)	M
1.00	PHE1(+1) CA+2(+1)	G
9.40	PHE1(+1) CA+2(+1) H +1(+1)	G
1.60	PHE1(+2) CA+2(+1)	G
18.60	PHE1(+2) CA+2(+1) H +1(+2)	G
7.59	PHE1(+1) CU+2(+1)	M
14.19	PHE1(+2) CU+2(+1)	M
3.15	PHE1(+1) FE+2(+1)	A
5.80	PHE1(+2) FE+2(+1)	A
8.85	PHE1(+1) FE+3(+1)	A
16.00	PHE1(+2) FE+3(+1)	A
21.00	PHE1(+3) FE+3(+1)	G

4.00	PHE1(+1)	PB+2(+1)		G
7.20	PHE1(+2)	PB+2(+1)		G
8.00	PHE1(+1)	PB+2(+1)	OH-1(+1)	G
1.70	PHE1(+1)	MG+2(+1)		G
9.80	PHE1(+1)	MG+2(+1)	H +1(+1)	G
2.30	PHE1(+2)	MG+2(+1)		G
19.00	PHE1(+2)	MG+2(+1)	H +1(+2)	G
2.40	PHE1(+1)	MN+2(+1)		A
4.30	PHE1(+2)	MN+2(+1)		A
5.10	PHE1(+1)	NI+2(+1)		A
9.00	PHE1(+2)	NI+2(+1)		A
4.21	PHE1(+1)	ZN+2(+1)		M
8.17	PHE1(+2)	ZN+2(+1)		M
10.25	PRO1(+1)	H +1(+1)		M
12.25	PRO1(+1)	H +1(+2)		M
1.30	PRO1(+1)	CA+2(+1)		G
11.50	PRO1(+1)	CA+2(+1)	H +1(+1)	G
1.70	PRO1(+2)	CA+2(+1)		G
21.80	PRO1(+2)	CA+2(+1)	H +1(+2)	G
8.68	PRO1(+1)	CU+2(+1)		M
16.00	PRO1(+2)	CU+2(+1)		M
10.64	PRO1(+1)	CU+2(+1)	H +1(+1)	M
4.00	PRO1(+1)	FE+2(+1)		G
7.00	PRO1(+2)	FE+2(+1)		E
9.69	PRO1(+1)	FE+3(+1)		A
17.90	PRO1(+2)	FE+3(+1)		G
4.00	PRO1(+1)	PB+2(+1)		G
6.00	PRO1(+2)	PB+2(+1)		G
8.00	PRO1(+1)	PB+2(+1)	OH-1(+1)	G
1.90	PRO1(+1)	MG+2(+1)		G
11.60	PRO1(+1)	MG+2(+1)	H +1(+1)	G
2.40	PRO1(+2)	MG+2(+1)		G
22.20	PRO1(+2)	MG+2(+1)	H +1(+2)	G
2.84	PRO1(+1)	MN+2(+1)		M
5.53	PRO1(+2)	MN+2(+1)		M
6.74	PRO1(+3)	MN+2(+1)		M
11.84	PRO1(+1)	MN+2(+1)	H +1(+1)	M
14.92	PRO1(+2)	MN+2(+1)	H +1(+1)	M
5.80	PRO1(+1)	NI+2(+1)		A
10.50	PRO1(+2)	NI+2(+1)		A
5.50	PRO1(+1)	ZN+2(+1)		M
9.90	PRO1(+2)	ZN+2(+1)		M
11.16	PRO1(+3)	ZN+2(+1)		M
12.52	PRO1(+1)	ZN+2(+1)	H +1(+1)	M
-2.60	PRO1(+1)	ZN+2(+1)	H +1(-1)	M
-0.06	PRO1(+2)	ZN+2(+1)	H +1(-1)	M
8.71	SER1(+1)	H +1(+1)		M
10.79	SER1(+1)	H +1(+2)		M
1.30	SER1(+1)	CA+2(+1)		A
9.50	SER1(+1)	CA+2(+1)	H +1(+1)	G
1.70	SER1(+2)	CA+2(+1)		G
18.60	SER1(+2)	CA+2(+1)	H +1(+2)	G
8.03	SER1(+1)	CU+2(+1)		M
14.37	SER1(+2)	CU+2(+1)		M
10.65	SER1(+1)	CU+2(+1)	H +1(+1)	M

4.83	SER1(+2) CU+2(+1) H +1(-1)	M
3.35	SER1(+1) FE+2(+1)	A
6.00	SER1(+2) FE+2(+1)	A
8.00	SER1(+3) FE+2(+1)	A
8.70	SER1(+1) FE+3(+1)	G
15.90	SER1(+2) FE+3(+1)	A
4.40	SER1(+1) PB+2(+1)	A
7.20	SER1(+2) PB+2(+1)	A
8.30	SER1(+3) PB+2(+1)	G
-3.50	SER1(+1) PB+2(+1) H +1(-1)	E
1.80	SER1(+1) MG+2(+1)	G
9.80	SER1(+1) MG+2(+1) H +1(+1)	G
2.40	SER1(+2) MG+2(+1)	G
18.90	SER1(+2) MG+2(+1) H +1(+2)	G
2.48	SER1(+1) MN+2(+1)	A
4.00	SER1(+2) MN+2(+1)	A
5.01	SER1(+1) NI+2(+1)	A
9.09	SER1(+2) NI+2(+1)	A
12.49	SER1(+3) NI+2(+1)	A
4.39	SER1(+1) ZN+2(+1)	M
8.17	SER1(+2) ZN+2(+1)	M
-1.14	SER1(+2) ZN+2(+1) H +1(-1)	M
10.56	SER1(+3) ZN+2(+1)	M
8.71	THR1(+1) H +1(+1)	M
10.91	THR1(+1) H +1(+2)	M
1.10	THR1(+1) CA+2(+1)	A
9.50	THR1(+1) CA+2(+1) H +1(+1)	G
1.40	THR1(+2) CA+2(+1)	G
18.50	THR1(+2) CA+2(+1) H +1(+2)	G
7.79	THR1(+1) CU+2(+1)	M
14.30	THR1(+2) CU+2(+1)	M
11.00	THR1(+1) CU+2(+1) H +1(+1)	E
1.60	THR1(+1) CU+2(+1) H +1(-1)	M
4.69	THR1(+2) CU+2(+1) H +1(-1)	M
3.50	THR1(+1) FE+2(+1)	A
6.00	THR1(+2) FE+2(+1)	A
8.40	THR1(+1) FE+3(+1)	A
15.40	THR1(+2) FE+3(+1)	G
4.40	THR1(+1) PB+2(+1)	G
7.40	THR1(+2) PB+2(+1)	G
8.00	THR1(+1) PB+2(+1) OH-1(+1)	G
1.70	THR1(+1) MG+2(+1)	G
9.80	THR1(+1) MG+2(+1) H +1(+1)	G
2.30	THR1(+2) MG+2(+1)	G
18.70	THR1(+2) MG+2(+1) H +1(+2)	G
2.56	THR1(+1) MN+2(+1)	A
3.90	THR1(+2) MN+2(+1)	A
5.30	THR1(+1) NI+2(+1)	A
9.40	THR1(+2) NI+2(+1)	A
12.40	THR1(+3) NI+2(+1)	A
4.83	THR1(+1) ZN+2(+1)	M
8.28	THR1(+2) ZN+2(+1)	M
-1.16	THR1(+2) ZN+2(+1) H +1(-1)	M
10.09	THR1(+3) ZN+2(+1)	M
9.07	TRP1(+1) H +1(+1)	M

11.50	TRP1(+1)	H +1(+2)	M
1.20	TRP1(+1)	CA+2(+1)	G
9.70	TRP1(+1)	CA+2(+1) H +1(+1)	G
1.60	TRP1(+2)	CA+2(+1)	G
19.10	TRP1(+2)	CA+2(+1) H +1(+2)	G
8.05	TRP1(+1)	CU+2(+1)	M
15.29	TRP1(+2)	CU+2(+1)	M
3.45	TRP1(+1)	FE+2(+1)	A
6.20	TRP1(+2)	FE+2(+1)	A
7.50	TRP1(+3)	FE+2(+1)	A
8.40	TRP1(+1)	FE+3(+1)	A
16.15	TRP1(+2)	FE+3(+1)	G
4.40	TRP1(+1)	PB+2(+1)	A
8.50	TRP1(+2)	PB+2(+1)	E
8.00	TRP1(+1)	PB+2(+1) OH-1(+1)	G
1.80	TRP1(+1)	MG+2(+1)	G
10.10	TRP1(+1)	MG+2(+1) H +1(+1)	G
2.30	TRP1(+2)	MG+2(+1)	G
19.50	TRP1(+2)	MG+2(+1) H +1(+2)	G
2.60	TRP1(+1)	MN+2(+1)	A
4.30	TRP1(+2)	MN+2(+1)	A
5.70	TRP1(+3)	MN+2(+1)	A
5.50	TRP1(+1)	NI+2(+1)	A
9.50	TRP1(+2)	NI+2(+1)	A
13.00	TRP1(+3)	NI+2(+1)	A
4.50	TRP1(+1)	ZN+2(+1)	M
8.76	TRP1(+2)	ZN+2(+1)	M
11.61	TRP1(+3)	ZN+2(+1)	M
10.06	TYR2(+1)	H +1(+1)	M
19.00	TYR2(+1)	H +1(+2)	M
21.35	TYR2(+1)	H +1(+3)	M
1.00	TYR2(+1)	CA+2(+1)	G
1.20	TYR2(+2)	CA+2(+1)	G
11.20	TYR2(+1)	CA+2(+1) H +1(+1)	A
19.50	TYR2(+1)	CA+2(+1) H +1(+2)	M
20.60	TYR2(+2)	CA+2(+1) H +1(+2)	M
38.80	TYR2(+2)	CA+2(+1) H +1(+4)	M
9.32	TYR2(+1)	CU+2(+1)	M
15.09	TYR2(+2)	CU+2(+1)	M
17.68	TYR2(+1)	CU+2(+1) H +1(+1)	M
25.08	TYR2(+2)	CU+2(+1) H +1(+1)	M
34.44	TYR2(+2)	CU+2(+1) H +1(+2)	M
3.00	TYR2(+1)	FE+2(+1)	G
13.50	TYR2(+1)	FE+2(+1) H +1(+1)	G
19.00	TYR2(+2)	FE+2(+1) H +1(+1)	G
27.00	TYR2(+2)	FE+2(+1) H +1(+2)	A
7.00	TYR2(+1)	FE+3(+1)	G
18.00	TYR2(+1)	FE+3(+1) H +1(+1)	G
28.24	TYR2(+2)	FE+3(+1) H +1(+1)	G
35.07	TYR2(+2)	FE+3(+1) H +1(+2)	G
3.50	TYR2(+1)	PB+2(+1)	G
14.16	TYR2(+1)	PB+2(+1) H +1(+1)	G
28.17	TYR2(+2)	PB+2(+1) H +1(+2)	G
21.00	TYR2(+2)	PB+2(+1) H +1(+1)	G
1.10	TYR2(+1)	MG+2(+1)	G

1.40	TYR2(+2)	MG+2(+1)		G
11.70	TYR2(+1)	MG+2(+1)	H +1(+1)	G
19.80	TYR2(+1)	MG+2(+1)	H +1(+2)	G
22.10	TYR2(+2)	MG+2(+1)	H +1(+2)	G
39.10	TYR2(+2)	MG+2(+1)	H +1(+4)	G
1.20	TYR2(+1)	MU+2(+1)		G
12.36	TYR2(+1)	MN+2(+1)	H +1(+1)	E
19.28	TYR2(+2)	MN+2(+1)	H +1(+1)	E
23.90	TYR2(+2)	MN+2(+1)	H +1(+2)	E
14.90	TYR2(+1)	NI+2(+1)	H +1(+1)	A
29.00	TYR2(+2)	NI+2(+1)	H +1(+2)	E
6.08	TYR2(+1)	ZN+2(+1)		A
14.27	TYR2(+1)	ZN+2(+1)	H +1(+1)	A
27.90	TYR2(+2)	ZN+2(+1)	H +1(+2)	A
21.00	TYR2(+2)	ZN+2(+1)	H +1(+1)	A
9.26	VAL1(+1)	H +1(+1)		M
11.66	VAL1(+1)	H +1(+2)		M
1.10	VAL1(+1)	CA+2(+1)		A
9.80	VAL1(+1)	CA+2(+1)	H +1(+1)	G
1.40	VAL1(+2)	CA+2(+1)		G
19.40	VAL1(+2)	CA+2(+1)	H +1(+2)	G
7.93	VAL1(+1)	CU+2(+1)		M
14.60	VAL1(+2)	CU+2(+1)		M
10.28	VAL1(+1)	CU+2(+1)	H +1(+1)	M
18.38	VAL1(+2)	CU+2(+1)	H +1(+1)	M
3.25	VAL1(+1)	FE+2(+1)		A
5.20	VAL1(+2)	FE+2(+1)		G
9.20	VAL1(+1)	FE+3(+1)		A
16.20	VAL1(+2)	FE+3(+1)		G
3.80	VAL1(+1)	PB+2(+1)		E
5.60	VAL1(+2)	PB+2(+1)		E
8.80	VAL1(+2)	PB+2(+1)	OH-1(+1)	E
1.70	VAL1(+1)	MG+2(+1)		G
10.20	VAL1(+1)	MG+2(+1)	H +1(+1)	G
2.20	VAL1(+2)	MG+2(+1)		G
19.80	VAL1(+2)	MG+2(+1)	H +1(+2)	G
2.337	VAL1(+1)	MN+2(+1)		M
3.97	VAL1(+2)	MN+2(+1)		M
5.19	VAL1(+3)	MN+2(+1)		M
10.48	VAL1(+1)	MN+2(+1)	H +1(+1)	M
12.73	VAL1(+2)	MN+2(+1)	H +1(+1)	M
5.15	VAL1(+1)	NI+2(+1)		A
9.45	VAL1(+2)	NI+2(+1)		A
11.30	VAL1(+3)	NI+2(+1)		A
4.44	VAL1(+1)	ZN+2(+1)		M
8.24	VAL1(+2)	ZN+2(+1)		M
10.62	VAL1(+3)	ZN+2(+1)		M
-4.18	VAL1(+1)	ZN+2(+1)	H +1(-1)	M
10.24	CO32(+1)	H +1(+1)		A
16.54	CO32(+1)	H +1(+2)		A
2.90	CO32(+1)	CA+2(+1)		A
10.90	CO32(+1)	CA+2(+1)	H +1(+1)	A
5.50	CO32(+1)	CU+2(+1)		A
8.30	CO32(+2)	CU+2(+1)		A
13.00	CO32(+1)	CU+2(+1)	H +1(+1)	G

14.20	CO32(+2)	CU+2(+1)	H +1(+2)	G
4.50	CO32(+1)	FE+2(+1)		A
6.00	CO32(+2)	FE+2(+1)		A
12.00	CO32(+1)	FE+2(+1)	H +1(+1)	A
13.00	CO32(+2)	FE+2(+1)	H +1(+2)	A
6.00	CO32(+1)	FE+3(+1)		A
9.00	CO32(+2)	FE+3(+1)		A
13.00	CO32(+1)	FE+3(+1)	H +1(+1)	A
14.50	CO32(+2)	FE+3(+1)	H +1(+2)	A
5.80	CO32(+1)	PB+2(+1)		A
8.80	CO32(+2)	PB+2(+1)		A
14.00	CO32(+1)	PB+2(+1)	H +1(+1)	G
14.90	CO32(+2)	PB+2(+1)	H +1(+2)	E
15.30	CO32(+3)	PB+2(+1)	H +1(+3)	A
2.90	CO32(+1)	MG+2(+1)		A
10.70	CO32(+1)	MG+2(+1)	H +1(+1)	A
3.10	CO32(+1)	MN+2(+1)		A
11.50	CO32(+1)	MN+2(+1)	H +1(+1)	A
3.30	CO32(+1)	ZN+2(+1)		A
11.80	CO32(+1)	ZN+2(+1)	H +1(+1)	A
-11.7	H +1(-1)	CA+2(+1)		M
-7.6	H +1(-1)	CU+2(+1)		M
-10.00	H +1(-2)	CU+2(+2)		M
-9.50	H +1(-1)	FE+2(+1)		A
-2.30	H +1(-1)	FE+3(+1)		A
-6.00	H +1(-2)	FE+3(+1)		A
-3.00	H +1(-2)	FE+3(+2)		A
-8.50	H +1(-1)	PB+2(+1)		A
-17.50	H +1(-2)	PB+2(+1)		A
-20.0	H +1(-1)	PB+2(+4)		A
-11.5	H +1(-1)	MG+2(+1)		M
-40.0	H +1(-4)	MG+2(+4)		A
-10.1	H +1(-1)	MN+2(+1)		M
-10.0	H +1(-1)	NI+2(+1)		A
-28.0	H +1(-4)	NI+2(+4)		A
-9.03	H +1(-1)	ZN+2(+1)		M
-17.00	H +1(-2)	ZN+2(+1)		A
-8.00	H +1(-1)	ZN+2(+2)		A
11.30	PO43(+1)	H +1(+1)		M
18.00	PO43(+1)	H +1(+2)		M
19.92	PO43(+1)	H +1(+3)		M
39.50	PO43(+2)	H +1(+5)		M
36.72	PO43(+2)	H +1(+4)		M
29.68	PO43(+2)	H +1(+3)		M
6.05	PO43(+1)	CA+2(+1)		M
12.70	PO43(+1)	CA+2(+1)	H +1(+1)	M
18.60	PO43(+1)	CA+2(+1)	H +1(+2)	M
16.30	PO43(+2)	CA+2(+2)	H +1(+2)	M
32.30	PO43(+2)	CA+2(+1)	H +1(+3)	M
25.30	PO43(+2)	CA+2(+1)	H +1(+2)	M
14.60	PO43(+1)	CU+2(+1)	H +1(+1)	M
19.30	PO43(+1)	CU+2(+1)	H +1(+2)	M
34.00	PO43(+2)	CU+2(+1)	H +1(+3)	M
31.80	PO43(+2)	CU+2(+2)	H +1(+2)	M
27.00	PO43(+2)	CU+2(+1)	H +1(+2)	M

13.50	PO43(+1) FE+2(+1) H +1(+1)	G
26.00	PO43(+2) FE+2(+1) H +1(+2)	G
17.00	PO43(+1) FE+3(+1) H +1(+1)	G
21.00	PO43(+1) FE+3(+1) H +1(+2)	G
28.00	PO43(+2) FE+3(+1) H +1(+2)	G
14.00	PO43(+1) PB+2(+1) H +1(+1)	A
26.30	PO43(+2) PB+2(+1) H +1(+2)	G
3.45	PO43(+1) MG+2(+1)	M
13.20	PO43(+1) MG+2(+1) H +1(+1)	M
18.70	PO43(+1) MG+2(+1) H +1(+2)	M
32.30	PO43(+2) MG+2(+1) H +1(+3)	M
27.60	PO43(+2) MG+2(+2) H +1(+2)	M
25.30	PO43(+2) MG+2(+1) H +1(+2)	M
13.30	PO43(+1) MN+2(+1) H +1(+1)	M
25.60	PO43(+2) MN+2(+1) H +1(+2)	M
13.70	PO43(+1) ZN+2(+1) H +1(+1)	M
19.20	PO43(+1) ZN+2(+1) H +1(+2)	M
33.30	PO43(+2) ZN+2(+1) H +1(+3)	M
31.00	PO43(+2) ZN+2(+2) H +1(+2)	M
26.30	PO43(+2) ZN+2(+1) H +1(+2)	M
11.40	SIL2(+1) H +1(+1)	A
20.50	SIL2(+1) H +1(+2)	A
3.00	SIL2(+1) CA+2(+1)	G
11.79	SIL2(+1) CA+2(+1) H +1(+1)	G
25.69	SIL2(+2) CA+2(+1) H +1(+2)	G
20.40	SIL2(+1) FE+3(+1) H +1(+1)	G
4.17	SIL2(+1) MG+2(+1)	G
12.04	SIL2(+1) MG+2(+1) H +1(+1)	G
26.62	SIL2(+2) MG+2(+1) H +1(+2)	G
1.60	SO42(+1) H +1(+1)	A
1.20	SO42(+1) CA+2(+1)	A
2.20	SO42(+1) CU+2(+1)	A
1.00	SO42(+1) FE+2(+1)	A
2.50	SO42(+1) FE+3(+1)	A
2.50	SO42(+1) FE+3(+1) H +1(+1)	A
2.60	SO42(+2) FE+3(+1)	A
3.00	SO42(+1) PB+2(+1)	A
3.50	SO42(+2) PB+2(+1)	A
1.50	SO42(+1) MG+2(+1)	A
2.00	SO42(+1) MN+2(+1)	A
2.20	SO42(+1) ZN+2(+1)	A
0.50	SCN1(+1) H +1(+1)	A
2.00	SCN1(+1) CU+2(+1)	A
2.40	SCN1(+2) CU+2(+1)	A
0.40	SCN1(+1) FE+2(+1)	A
2.30	SCN1(+1) FE+3(+1)	A
3.30	SCN1(+2) FE+3(+1)	A
1.00	SCN1(+1) PB+2(+1)	A
1.50	SCN1(+2) PB+2(+1)	G
1.05	SCN1(+1) MN+2(+1)	A
1.20	SCN1(+1) ZN+2(+1)	A
1.60	SCN1(+2) ZN+2(+1)	A
2.00	SCN1(+3) ZN+2(+1)	A
2.40	SCN1(+4) ZN+2(+1)	G
8.95	NH30(+1) H +1(+1)	A

3.90	NH30(+1)	CU+2(+1)		A
7.13	NH30(+2)	CU+2(+1)		A
9.90	NH30(+3)	CU+2(+1)		A
11.90	NH30(+4)	CU+2(+1)		A
1.30	NH30(+1)	FE+2(+1)		A
1.90	NH30(+2)	FE+2(+1)		A
3.00	NH30(+4)	FE+2(+1)		A
0.50	NH30(+1)	MN+2(+1)		A
2.50	NH30(+1)	NI+2(+1)		A
4.50	NH30(+2)	NI+2(+1)		A
6.00	NH30(+3)	NI+2(+1)		A
7.00	NH30(+4)	NI+2(+1)		G
7.70	NH30(+5)	NI+2(+1)		G
8.00	NH30(+6)	NI+2(+1)		G
2.25	NH30(+1)	ZN+2(+1)		A
4.55	NH30(+2)	ZN+2(+1)		A
6.75	NH30(+3)	ZN+2(+1)		A
9.01	NH30(+4)	ZN+2(+1)		A
15.50	NH30(+1)	CU+2(+1)	OH-1(+3)	A
15.00	NH30(+2)	CU+2(+1)	OH-1(+2)	A
14.50	NH30(+3)	CU+2(+1)	OH-1(+1)	A
14.00	NH30(+1)	ZN+2(+1)	OH-1(+3)	A
13.10	NH30(+2)	ZN+2(+1)	OH-1(+2)	A
11.50	NH30(+3)	ZN+2(+1)	OH-1(+1)	A
10.30	NH30(+2)	ZN+2(+1)	OH-1(+1)	A
8.80	NH30(+1)	ZN+2(+1)	OH-1(+1)	A
10.35	ACA2(+1)	H +1(+1)		M
14.31	ACA2(+1)	H +1(+2)		M
10.45	ACA2(+1)	CA+2(+1)	H +1(+1)	A
11.35	ACA2(+1)	CU+2(+1)	H +1(+1)	A
6.00	ACA2(+1)	FE+2(+1)		M
10.55	ACA2(+1)	FE+2(+1)	H +1(+1)	A
11.00	ACA2(+1)	PB+2(+1)	H +1(+1)	A
10.45	ACA2(+1)	MG+2(+1)	H +1(+1)	G
10.80	ACA2(+1)	MN+2(+1)	H +1(+1)	G
11.00	ACA2(+1)	ZN+2(+1)	H +1(+1)	G
5.504	CTA3(+1)	H +1(+1)		M
9.725	CTA3(+1)	H +1(+2)		M
12.54	CTA3(+1)	H +1(+3)		M
3.26	CTA3(+1)	CA+2(+1)		M
4.00	CTA3(+2)	CA+2(+1)		A
7.53	CTA3(+1)	CA+2(+1)	H +1(+1)	M
10.95	CTA3(+1)	CA+2(+1)	H +1(+2)	A
16.00	CTA3(+2)	CA+2(+1)	H +1(+2)	A
5.50	CTA3(+1)	CU+2(+1)		A
7.80	CTA3(+2)	CU+2(+1)		A
8.00	CTA3(+1)	CU+2(+1)	H +1(+1)	A
1.00	CTA3(+1)	CU+2(+1)	H +1(-1)	G
9.30	CTA3(+2)	CU+2(+1)	H +1(+1)	G
14.00	CTA3(+2)	CU+2(+1)	H +1(+2)	G
4.00	CTA3(+1)	FE+2(+1)		A
8.30	CTA3(+1)	FE+2(+1)	H +1(+1)	A
6.00	CTA3(+2)	FE+2(+1)		M
-4.00	CTA3(+1)	FE+2(+1)	H +1(-1)	M
10.50	CTA3(+1)	FE+3(+1)		A

12.08	CTA3(+1) FE+3(+1) H +1(+1)	G
16.00	CTA3(+2) FE+3(+1)	A
17.00	CTA3(+2) FE+3(+1) H +1(+1)	M
23.00	CTA3(+2) FE+3(+1) H +1(+2)	M
9.00	CTA3(+1) FE+3(+1) H +1(-1)	A
1.50	CTA3(+1) FE+3(+1) H +1(-2)	A
3.00	CTA3(+2) FE+3(+1) H +1(-2)	A
18.56	CTA3(+2) FE+3(+2) H +1(-2)	M
4.30	CTA3(+1) PB+2(+1)	A
8.00	CTA3(+1) PB+2(+1) H +1(+1)	A
10.50	CTA3(+1) PB+2(+1) H +1(+2)	A
-4.00	CTA3(+1) PB+2(+1) H +1(-1)	A
5.40	CTA3(+2) PB+2(+1)	A
10.00	CTA3(+2) PB+2(+1) H +1(+1)	A
14.30	CTA3(+2) PB+2(+1) H +1(+2)	A
17.90	CTA3(+2) PB+2(+1) H +1(+3)	A
20.50	CTA3(+2) PB+2(+1) H +1(+4)	A
2.00	CTA3(+3) PB+2(+1)	A
6.00	CTA3(+1) PB+2(+2)	A
14.00	CTA3(+2) PB+2(+2) H +1(+1)	E
4.00	CTA3(+2) PB+2(+2) H +1(-1)	E
-3.50	CTA3(+2) PB+2(+2) H +1(-2)	E
11.50	CTA3(+3) PB+2(+2)	E
1.00	CTA3(+2) PB+2(+3) H +1(-2)	E
8.50	CTA3(+3) PB+2(+4) H +1(-2)	E
3.34	CTA3(+1) MG+2(+1)	M
4.10	CTA3(+2) MG+2(+1)	A
7.32	CTA3(+1) MG+2(+1) H +1(+1)	M
10.00	CTA3(+1) MG+2(+1) H +1(+2)	G
3.82	CTA3(+1) MN+2(+1)	M
4.30	CTA3(+2) MN+2(+1)	G
5.00	CTA3(+1) MN+2(+1) H +1(+1)	A
-5.50	CTA3(+1) MN+2(+1) H +1(-1)	G
4.90	CTA3(+1) NI+2(+1)	A
8.30	CTA3(+1) NI+2(+1) H +1(+1)	A
5.50	CTA3(+2) NI+2(+1) H +1(+2)	A
-1.00	CTA3(+1) NI+2(+1) H +1(-1)	G
4.715	CTA3(+1) ZN+2(+1)	M
8.44	CTA3(+1) ZN+2(+1) H +1(+1)	M
10.95	CTA3(+1) ZN+2(+1) H +1(+2)	M
-2.50	CTA3(+1) ZN+2(+1) H +1(-1)	M
7.36	CTA3(+2) ZN+2(+1)	M
-2.21	CTA3(+2) ZN+2(+2) H +1(-2)	M
3.78	LTA1(+1) H +1(+1)	A
1.30	LTA1(+1) CA+2(+1)	A
2.00	LTA1(+2) CA+2(+1)	A
2.20	LTA1(+1) CU+2(+1)	A
3.00	LTA1(+2) CU+2(+1)	A
3.03	LTA1(+3) CU+2(+1)	A
1.50	LTA1(+1) FE+2(+1)	G
4.00	LTA1(+1) FE+3(+1)	G
6.00	LTA1(+2) FE+3(+1)	E
1.75	LTA1(+1) PB+2(+1)	A
2.50	LTA1(+2) PB+2(+1)	A
1.40	LTA1(+1) MG+2(+1)	A

1.80	LTA1(+2)	MG+2(+1)	A
1.00	LTA1(+1)	MN+2(+1)	A
1.40	LTA1(+2)	MN+2(+1)	A
1.40	LTA1(+1)	NI+2(+1)	A
2.40	LTA1(+2)	NI+2(+1)	A
2.60	LTA1(+3)	NI+2(+1)	A
1.75	LTA1(+1)	ZN+2(+1)	A
2.75	LTA1(+2)	ZN+2(+1)	A
4.48	MLA2(+1)	H +1(+1)	M
7.59	MLA2(+1)	H +1(+2)	M
1.80	MLA2(+1)	CA+2(+1)	A
2.40	MLA2(+2)	CA+2(+1)	G
5.50	MLA2(+1)	CA+2(+1) H +1(+1)	A
3.40	MLA2(+1)	CU+2(+1)	A
5.50	MLA2(+2)	CU+2(+1)	A
7.20	MLA2(+1)	CU+2(+1) H +1(+1)	A
2.40	MLA2(+1)	FE+2(+1)	A
3.60	MLA2(+2)	FE+2(+1)	G
6.00	MLA2(+1)	FE+2(+1) H +1(+1)	G
6.70	MLA2(+1)	FE+3(+1)	A
11.50	MLA2(+2)	FE+3(+1)	G
0.00	MLA2(+1)	FE+3(+1) H +1(-1)	G
12.00	MLA2(+2)	FE+3(+2) H +1(-2)	G
2.90	MLA2(+1)	PB+2(+1)	G
3.40	MLA2(+2)	PB+2(+1)	G
-4.00	MLA2(+1)	PB+2(+1) H +1(-1)	G
1.50	MLA2(+1)	MG+2(+1)	A
2.00	MLA2(+2)	MG+2(+1)	G
5.60	MLA2(+1)	MG+2(+1) H +1(+1)	A
2.00	MLA2(+1)	MN+2(+1)	A
3.00	MLA2(+2)	MN+2(+1)	G
5.70	MLA2(+1)	MN+2(+1) H +1(+1)	G
3.00	MLA2(+1)	NI+2(+1)	G
4.80	MLA2(+2)	NI+2(+1)	G
6.50	MLA2(+1)	NI+2(+1) H +1(+1)	G
2.90	MLA2(+1)	ZN+2(+1)	M
4.50	MLA2(+2)	ZN+2(+1)	G
6.24	MLA2(+1)	ZN+2(+1) H +1(+1)	M
-3.64	MLA2(+1)	ZN+2(+1) H +1(-1)	M
3.682	OXA2(+1)	H +1(+1)	M
4.768	OXA2(+1)	H +1(+2)	M
1.80	OXA2(+1)	CA+2(+1)	A
2.40	OXA2(+2)	CA+2(+1)	A
4.70	OXA2(+1)	CA+2(+1) H +1(+1)	A
4.80	OXA2(+1)	CU+2(+1)	A
8.20	OXA2(+2)	CU+2(+1)	A
6.00	OXA2(+1)	CU+2(+1) H +1(+1)	A
3.50	OXA2(+1)	FE+2(+1)	A
6.00	OXA2(+2)	FE+2(+1)	A
5.30	OXA2(+1)	FE+2(+1) H +1(+1)	G
8.00	OXA2(+1)	FE+3(+1)	A
14.20	OXA2(+2)	FE+3(+1)	A
19.00	OXA2(+3)	FE+3(+1)	A
7.80	OXA2(+1)	FE+3(+1) H +1(+1)	A
2.00	OXA2(+1)	FE+3(+1) H +1(-1)	G

14.00	OXA2(+2) FE+3(+2) H +1(-2)		G
3.90	OXA2(+1) PB+2(+1)		A
5.70	OXA2(+2) PB+2(+1)		A
-3.00	OXA2(+1) PB+2(+1) H +1(-1)		G
2.40	OXA2(+1) MG+2(+1)		A
3.80	OXA2(+2) MG+2(+1)		A
4.80	OXA2(+1) MG+2(+1) H +1(+1)		G
3.50	OXA2(+1) MN+2(+1)		A
4.80	OXA2(+2) MN+2(+1)		A
5.00	OXA2(+1) MN+2(+1) H +1(+1)		A
5.00	OXA2(+1) NI+2(+1)		A
7.40	OXA2(+2) NI+2(+1)		A
5.60	OXA2(+1) NI+2(+1) H +1(+1)		G
4.05	OXA2(+1) ZN+2(+1)		M
6.60	OXA2(+2) ZN+2(+1)		A
7.10	OXA2(+3) ZN+2(+1)		A
5.00	OXA2(+1) ZN+2(+1) H +1(+1)		A
7.20	OXA2(+2) ZN+2(+1) H +1(+2)		A
-2.00	OXA2(+1) ZN+2(+1) H +1(-1)		G
2.30	PVA1(+1) H +1(+1)		A
0.75	PVA1(+1) CA+2(+1)		A
2.20	PVA1(+1) CU+2(+1)		A
4.20	PVA1(+2) CU+2(+1)		A
1.20	PVA1(+1) FE+2(+1)		G
1.90	PVA1(+2) FE+2(+1)		G
4.00	PVA1(+1) FE+3(+1)		G
6.00	PVA1(+2) FE+3(+1)		G
1.50	PVA1(+1) PB+2(+1)		G
2.80	PVA1(+2) PB+2(+1)		A
0.75	PVA1(+1) MG+2(+1)		G
1.00	PVA1(+1) MN+2(+1)		A
1.00	PVA1(+1) NI+2(+1)		A
1.10	PVA1(+2) NI+2(+1)		G
1.50	PVA1(+1) ZN+2(+1)		G
2.20	PVA1(+2) ZN+2(+1)		A
13.000	SLA2(+1) H +1(+1)		A
15.751	SLA2(+1) H +1(+2)		M
4.29	SLA2(+1) CA+2(+1)		M
7.5	SLA2(+2) CA+2(+1)		M
14.30	SLA2(+1) CA+2(+1) H +1(+1)		M
10.045	SLA2(+1) CU+2(+1)		M
17.023	SLA2(+2) CU+2(+1)		M
6.00	SLA2(+1) FE+2(+1)		A
10.00	SLA2(+2) FE+2(+1)		A
16.00	SLA2(+1) FE+3(+1)		A
27.00	SLA2(+2) FE+3(+1)		A
34.00	SLA2(+3) FE+3(+1)		A
17.00	SLA2(+1) FE+3(+1) H +1(+1)		A
5.50	SLA2(+1) PB+2(+1)		G
9.00	SLA2(+2) PB+2(+1)		G
5.15	SLA2(+1) MG+2(+1)		M
9.54	SLA2(+2) MG+2(+1)		M
13.9	SLA2(+1) MG+2(+1) H +1(+1)		M
5.50	SLA2(+1) MN+2(+1)		A
9.00	SLA2(+2) MN+2(+1)		A

2.10	SCA2(+2)	MG+2(+1)		A
5.80	SCA2(+1)	MG+2(+1)	H +1(+1)	A
2.71	SCA2(+1)	MN+2(+1)		A
5.44	SCA2(+2)	MN+2(+1)		A
7.41	SCA2(+1)	MN+2(+1)	H +1(+1)	A
2.00	SCA2(+1)	NI+2(+1)		A
3.50	SCA2(+2)	NI+2(+1)		A
2.96	SCA2(+1)	ZN+2(+1)		A
5.09	SCA2(+2)	ZN+2(+1)		G
7.46	SCA2(+1)	ZN+2(+1)	H +1(+1)	A
4.00	TRA2(+1)	H +1(+1)		A
6.80	TRA2(+1)	H +1(+2)		A
1.80	TRA2(+1)	CA+2(+1)		A
2.40	TRA2(+2)	CA+2(+1)		G
5.00	TRA2(+1)	CA+2(+1)	H +1(+1)	A
2.80	TRA2(+1)	CU+2(+1)		A
4.30	TRA2(+2)	CU+2(+1)		A
6.00	TRA2(+1)	CU+2(+1)	H +1(+1)	G
2.00	TRA2(+1)	FE+2(+1)		A
3.00	TRA2(+2)	FE+2(+1)		G
5.40	TRA2(+1)	FE+2(+1)	H +1(+1)	E
6.00	TRA2(+1)	FE+3(+1)		A
10.50	TRA2(+2)	FE+3(+1)		A
-1.00	TRA2(+1)	FE+3(+1)	H +1(-1)	G
11.00	TRA2(+2)	FE+3(+2)	H +1(-2)	E
2.60	TRA2(+1)	PB+2(+1)		A
3.00	TRA2(+2)	PB+2(+1)		G
-5.00	TRA2(+1)	PB+2(+1)	H +1(-1)	G
1.50	TRA2(+1)	MG+2(+1)		A
2.00	TRA2(+2)	MG+2(+1)		G
5.00	TRA2(+1)	MG+2(+1)	H +1(+1)	A
1.50	TRA2(+1)	MN+2(+1)		A
2.50	TRA2(+2)	MN+2(+1)		G
5.20	TRA2(+1)	MN+2(+1)	H +1(+1)	G
2.50	TRA2(+1)	NI+2(+1)		G
4.00	TRA2(+2)	NI+2(+1)		A
5.80	TRA2(+1)	NI+2(+1)	H +1(+1)	G
2.40	TRA2(+1)	ZN+2(+1)		A
3.90	TRA2(+2)	ZN+2(+1)		A
5.30	TRA2(+1)	ZN+2(+1)	H +1(+1)	A
-4.00	TRA2(+1)	ZN+2(+1)	H +1(-1)	G
10.30	PEN2(+1)	H +1(+1)		A
18.10	PEN2(+1)	H +1(+2)		A
20.40	PEN2(+1)	H +1(+3)		A
2.45	PEN2(+1)	CA+2(+1)		A
2.80	PEN2(+2)	CA+2(+1)		G
15.00	PEN2(+1)	CU+1(+1)		G
19.00	PEN2(+2)	CU+1(+1)		G
23.00	PEN2(+1)	CU+1(+1)	H +1(+1)	G
38.40	PEN2(+2)	CU+1(+1)	H +1(+2)	E
98.00	PEN2(+4)	CU+1(+5)		E
12.50	PEN2(+1)	PB+2(+1)		A
16.50	PEN2(+2)	PB+2(+1)		A
19.30	PEN2(+3)	PB+2(+1)		A
15.40	PEN2(+1)	PB+2(+1)	H +1(+1)	A

26.40	PEN2(+2)	PB+2(+1)	H +1(+1)	A
32.00	PEN2(+2)	PB+2(+1)	H +1(+2)	E
2.55	PEN2(+1)	MG+2(+1)		E
3.50	PEN2(+2)	MG+2(+1)		G
5.00	PEN2(+1)	MN+2(+1)		E
8.90	PEN2(+2)	MN+2(+1)		E
10.30	PEN2(+1)	NI+2(+1)		A
21.70	PEN2(+2)	NI+2(+1)		A
24.40	PEN2(+3)	NI+2(+1)		E
9.35	PEN2(+1)	ZN+2(+1)		A
18.70	PEN2(+2)	ZN+2(+1)		A
22.00	PEN2(+3)	ZN+2(+1)		A
8.00	PEN2(+2)	ZN+2(+1)	H +1(-1)	A
25.00	PEN2(+2)	ZN+2(+1)	H +1(+1)	A
30.50	PEN2(+2)	ZN+2(+1)	H +1(+2)	A
8.59	OPN2(+1)	H +1(+1)		M
16.23	OPN2(+1)	H +1(+2)		M
18.23	OPN2(+1)	H +1(+3)		M
19.57	OPN2(+1)	H +1(+4)		M
9.70	OPN2(+1)	CA+2(+1)	H +1(+1)	G
19.10	OPN2(+2)	CA+2(+1)	H +1(+2)	G
7.22	OPN2(+1)	CU+2(+1)		M
15.51	OPN2(+1)	CU+2(+1)	H +1(+1)	M
27.40	OPN2(+2)	CU+2(+2)		M
27.00	OPN2(+2)	CU+2(+1)	H +1(+2)	G
17.00	OPN2(+1)	FE+3(+1)	H +1(+1)	G
31.00	OPN2(+2)	FE+3(+1)	H +1(+2)	G
25.00	OPN2(+2)	FE+3(+1)	H +1(+1)	G
15.50	OPN2(+1)	PB+2(+1)	H +1(+1)	G
21.50	OPN2(+2)	PB+2(+1)	H +1(+2)	G
10.30	OPN2(+1)	MG+2(+1)	H +1(+1)	G
19.80	OPN2(+2)	MG+2(+1)	H +1(+2)	G
10.60	OPN2(+1)	MN+2(+1)	H +1(+1)	G
22.00	OPN2(+2)	MN+2(+1)	H +1(+2)	G
6.0	OPN2(+1)	NI+2(+1)		G
13.0	OPN2(+1)	NI+2(+1)	H +1(+1)	G
5.18	OPN2(+1)	ZN+2(+1)		M
11.73	OPN2(+1)	ZN+2(+1)	H +1(+1)	M
25.80	OPN2(+2)	ZN+2(+1)	H +1(+2)	G
9.289	GSH3(+1)	H +1(+1)		M
17.67	GSH3(+1)	H +1(+2)		M
21.11	GSH3(+1)	H +1(+3)		M
23.14	GSH3(+1)	H +1(+4)		M
3.84	GSH3(+1)	CA+2(+1)		G
4.00	GSH3(+2)	CA+2(+1)		G
12.89	GSH3(+1)	CA+2(+1)	H +1(+1)	G
20.68	GSH3(+1)	CA+2(+1)	H +1(+2)	G
15.00	GSH3(+1)	CU+1(+1)		M
19.00	GSH3(+2)	CU+1(+1)		M
24.50	GSH3(+1)	CU+1(+1)	H +1(+1)	M
38.00	GSH3(+2)	CU+1(+1)	H +1(+2)	M
6.00	GSH3(+1)	FE+2(+1)		G
9.00	GSH3(+2)	FE+2(+1)		G
10.00	GSH3(+1)	PB+2(+1)		G
14.00	GSH3(+2)	PB+2(+1)		G

16.40	GSH3(+1)	PB+2(+1) H +1(+1)	A
22.80	GSH3(+2)	PB+2(+1) H +1(+1)	A
29.50	GSH3(+2)	PB+2(+1) H +1(+2)	A
4.00	GSH3(+2)	PB+2(+1) H +1(-1)	A
4.00	GSH3(+1)	MG+2(+1)	G
4.20	GSH3(+2)	MG+2(+1)	G
5.00	GSH3(+1)	MN+2(+1)	A
6.00	GSH3(+2)	MN+2(+1)	G
7.80	GSH3(+1)	NI+2(+1)	M
13.00	GSH3(+2)	NI+2(+1)	M
7.98	GSH3(+1)	ZN+2(+1)	M
12.48	GSH3(+2)	ZN+2(+1)	M
14.11	GSH3(+1)	ZN+2(+1) H +1(+1)	M
21.36	GSH3(+2)	ZN+2(+1) H +1(+1)	M
28.08	GSH3(+2)	ZN+2(+1) H +1(+2)	M
3.12	GSH3(+2)	ZN+2(+1) H +1(-1)	M
09.18	GSS4(+1)	H +1(+1)	M
17.78	GSS4(+1)	H +1(+2)	M
21.71	GSS4(+1)	H +1(+3)	M
24.80	GSS4(+1)	H +1(+4)	M
27.50	GSS4(+1)	H +1(+5)	M
28.90	GSS4(+1)	H +1(+6)	M
13.76	GSS4(+1)	CU+2(+1)	M
17.48	GSS4(+2)	CU+2(+1)	M
16.05	GSS4(+1)	CU+2(+2)	M
17.71	GSS4(+1)	CU+2(+1) H +1(+1)	M
21.50	GSS4(+1)	CU+2(+1) H +1(+2)	M
28.00	GSS4(+2)	CU+2(+1) H +1(+2)	M
4.09	GSS4(+1)	CU+2(+1) OH-1(+1)	M
7.22	GSS4(+1)	ZN+2(+1)	M
10.00	GSS4(+2)	ZN+2(+1)	M
12.60	GSS4(+1)	ZN+2(+1) H +1(+1)	M
9.57	TETO(+1)	H +1(+1)	M
18.51	TETO(+1)	H +1(+2)	M
24.95	TETO(+1)	H +1(+3)	M
28.05	TETO(+1)	H +1(+4)	M
19.41	TETO(+1)	CU+2(+1)	M
22.03	TETO(+1)	CU+2(+1) H +1(+1)	M
24.95	TETO(+1)	CU+2(+1) H +1(+2)	M
22.50	TETO(+1)	CU+2(+1) OH-1(+1)	G
7.50	TETO(+1)	FE+2(+1)	A
21.30	TETO(+1)	FE+3(+1)	A
9.80	TETO(+1)	PB+2(+1)	A
4.70	TETO(+1)	MN+2(+1)	A
14.00	TETO(+1)	NI+2(+1)	A
13.00	TETO(+1)	NI+2(+1) H +1(+1)	A
11.19	TETO(+1)	ZN+2(+1)	M
15.87	TETO(+1)	ZN+2(+1) H +1(+1)	M
9.120	EDT4(+1)	H +1(+1)	A
15.03	EDT4(+1)	H +1(+2)	A
17.80	EDT4(+1)	H +1(+3)	A
19.90	EDT4(+1)	H +1(+4)	A
9.36	EDT4(+1)	CA+2(+1)	A
11.50	EDT4(+1)	CA+2(+1) H +1(+1)	A
10.50	EDT4(+1)	CA+2(+2)	A

17.60	EDT4(+1)	CU+2(+1)	A
20.80	EDT4(+1)	CU+2(+1) H +1(+1)	A
22.80	EDT4(+1)	CU+2(+1) H +1(+2)	A
19.00	EDT4(+1)	CU+2(+1) OH-1(+1)	A
13.50	EDT4(+1)	FE+2(+1)	A
16.00	EDT4(+1)	FE+2(+1) H +1(+1)	A
17.50	EDT4(+1)	FE+2(+1) OH-1(+1)	A
21.00	EDT4(+1)	FE+2(+1) OH-1(+2)	A
24.10	EDT4(+1)	FE+3(+1)	A
24.50	EDT4(+1)	FE+3(+1) H +1(+1)	A
30.50	EDT4(+1)	FE+3(+1) OH-1(+1)	A
33.50	EDT4(+1)	FE+3(+1) OH-1(+2)	A
16.70	EDT4(+1)	PB+2(+1)	A
20.00	EDT4(+1)	PB+2(+1) H +1(+1)	A
7.76	EDT4(+1)	MG+2(+1)	A
12.00	EDT4(+1)	MG+2(+1) H +1(+1)	A
13.00	EDT4(+1)	MN+2(+1)	A
16.00	EDT4(+1)	MN+2(+1) H +1(+1)	A
17.50	EDT4(+1)	NI+2(+1)	A
20.50	EDT4(+1)	NI+2(+1) H +1(+1)	A
20.00	EDT4(+1)	NI+2(+1) OH-1(+1)	A
15.50	EDT4(+1)	ZN+2(+1)	A
18.30	EDT4(+1)	ZN+2(+1) H +1(+1)	A
17.60	EDT4(+1)	ZN+2(+1) OH-1(+1)	A
7.97	GH 1(+1)	H +1(+1)	M
14.58	GH 1(+1)	H +1(+2)	M
17.24	GH 1(+1)	H +1(+3)	M
8.68	GH 1(+1)	CU+2(+1)	M
15.41	GH 1(+2)	CU+2(+1)	M
12.25	GH 1(+1)	CU+2(+1) H +1(+1)	M
4.54	GH 1(+1)	CU+2(+1) H +1(-1)	M
-4.94	GH 1(+1)	CU+2(+1) H +1(-2)	M
20.45	GH 1(+2)	CU+2(+1) H +1(+1)	M
7.68	GH 1(+2)	CU+2(+1) H +1(-1)	M
5.00	GH 1(+1)	NI+2(+1)	G
7.50	GH 1(+2)	NI+2(+1)	G
10.80	GH 1(+1)	NI+2(+1) H +1(+1)	G
-1.90	GH 1(+1)	NI+2(+1) H +1(-1)	A
-11.0	GH 1(+1)	NI+2(+1) H +1(-2)	E
3.65	GH 1(+1)	ZN+2(+1)	M
6.89	GH 1(+2)	ZN+2(+1)	M
10.00	GH 1(+1)	ZN+2(+1) H +1(+1)	A
-3.50	GH 1(+1)	ZN+2(+1) H +1(-1)	A
7.51	GGH1(+1)	H +1(+1)	M
14.03	GGH1(+1)	H +1(+2)	M
16.46	GGH1(+1)	H +1(+3)	M
7.04	GGH1(+1)	CU+2(+1)	M
11.70	GGH1(+1)	CU+2(+1) H +1(+1)	M
3.00	GGH1(+1)	CU+2(+1) H +1(-1)	A
-2.11	GGH1(+1)	CU+2(+1) H +1(-2)	M
8.70	GGH1(+2)	CU+2(+1) H +1(-1)	M
1.30	GGH1(+2)	CU+2(+1) H +1(-2)	M
4.20	GGH1(+1)	NI+2(+1)	G
10.50	GGH1(+1)	NI+2(+1) H +1(+1)	G
-2.00	GGH1(+1)	NI+2(+1) H +1(-1)	A

-8.50	GGH1(+1) NI+2(+1) H +1(-2)	A
3.31	GGH1(+1) ZN+2(+1)	M
10.08	GGH1(+1) ZN+2(+1) H +1(+1)	M
-4.00	GGH1(+1) ZN+2(+1) H +1(-1)	G
7.50	PMA0(+1) H +1(+1)	E
13.90	PMA0(+1) H +1(+2)	E
7.00	PMA0(+1) CU+2(+1)	G
3.00	PMA0(+1) CU+2(+1) H +1(-1)	G
-0.60	PMA0(+1) CU+2(+1) H +1(-2)	E
4.00	PMA0(+1) NI+2(+1)	G
10.00	PMA0(+1) NI+2(+1) H +1(+1)	G
-2.00	PMA0(+1) NI+2(+1) H +1(-1)	G
-7.50	PMA0(+1) NI+2(+1) H +1(-2)	G
3.50	PMA0(+1) ZN+2(+1)	G
10.00	PMA0(+1) ZN+2(+1) H +1(+1)	G
-4.00	PMA0(+1) ZN+2(+1) H +1(-1)	G
10.60	BAL2(+1) H +1(+1)	A
19.20	BAL2(+1) H +1(+2)	A
14.00	BAL2(+1) PB+2(+1)	G
20.50	BAL2(+2) PB+2(+1)	G
5.00	BAL2(+1) MN+2(+1)	E
9.95	BAL2(+2) MN+2(+1)	E
11.20	BAL2(+1) NI+2(+1)	G
22.50	BAL2(+2) NI+2(+1)	E
13.00	BAL2(+1) ZN+2(+1)	E
22.00	BAL2(+2) ZN+2(+1)	E
9.50	DFO2(+1) H +1(+1)	A
18.50	DFO2(+1) H +1(+2)	A
26.80	DFO2(+1) H +1(+3)	A
2.60	DFO2(+1) CA+2(+1)	A
14.00	DFO2(+1) CU+2(+1)	A
23.00	DFO2(+1) CU+2(+1) H +1(+1)	A
26.00	DFO2(+1) CU+2(+1) H +1(+2)	A
16.50	DFO2(+1) FE+2(+1) H +1(+1)	A
22.00	DFO2(+1) FE+2(+1) H +1(+2)	A
29.80	DFO2(+1) FE+3(+1)	A
31.00	DFO2(+1) FE+3(+1) H +1(+1)	A
20.00	DFO2(+1) FE+3(+1) H +1(-1)	A
4.20	DFO2(+1) MG+2(+1)	A
7.00	DFO2(+1) MN+2(+1)	A
10.80	DFO2(+1) NI+2(+1)	A
17.00	DFO2(+1) NI+2(+1) H +1(+1)	A
22.80	DFO2(+1) NI+2(+1) H +1(+2)	A
11.00	DFO2(+1) ZN+2(+1)	A
17.20	DFO2(+1) ZN+2(+1) H +1(+1)	A
22.90	DFO2(+1) ZN+2(+1) H +1(+2)	A
9.673	DTP5(+1) H +1(+1)	A
17.94	DTP5(+1) H +1(+2)	A
22.09	DTP5(+1) H +1(+3)	A
24.78	DTP5(+1) H +1(+4)	A
26.91	DTP5(+1) H +1(+5)	A
9.82	DTP5(+1) CA+2(+1)	A
15.80	DTP5(+1) CA+2(+1) H +1(+1)	A
20.22	DTP5(+1) CA+2(+1) H +1(+2)	A
23.92	DTP5(+1) CA+2(+1) H +1(+3)	A

11.92	DTP5(+1)	CA+2(+2)	A
20.30	DTP5(+1)	CU+2(+1)	A
25.00	DTP5(+1)	CU+2(+1) H +1(+1)	A
28.00	DTP5(+1)	CU+2(+1) H +1(+2)	A
15.50	DTP5(+1)	FE+2(+1)	A
20.70	DTP5(+1)	FE+2(+1) H +1(+1)	A
20.50	DTP5(+1)	FE+2(+1) OH-1(+1)	A
27.00	DTP5(+1)	FE+3(+1)	A
30.50	DTP5(+1)	FE+3(+1) H +1(+1)	A
31.20	DTP5(+1)	FE+3(+1) OH-1(+1)	A
17.50	DTP5(+1)	PB+2(+1)	A
22.60	DTP5(+1)	PB+2(+1) H +1(+1)	A
8.48	DTP5(+1)	MG+2(+1)	A
15.40	DTP5(+1)	MG+2(+1) H +1(+1)	A
19.95	DTP5(+1)	MG+2(+1) H +1(+2)	A
14.50	DTP5(+1)	MN+2(+1)	A
16.50	DTP5(+1)	MN+2(+1) H +1(+1)	A
19.00	DTP5(+1)	NI+2(+1)	A
24.00	DTP5(+1)	NI+2(+1) H +1(+1)	A
27.00	DTP5(+1)	NI+2(+1) H +1(+2)	A
17.00	DTP5(+1)	ZN+2(+1)	A
22.50	DTP5(+1)	ZN+2(+1) H +1(+1)	A
25.80	DTP5(+1)	ZN+2(+1) H +1(+2)	A
11.90	CDT4(+1)	H +1(+1)	A
17.90	CDT4(+1)	H +1(+2)	A
21.20	CDT4(+1)	H +1(+3)	A
23.60	CDT4(+1)	H +1(+4)	A
25.00	CDT4(+1)	H +1(+5)	A
12.00	CDT4(+1)	CA+2(+1)	A
21.70	CDT4(+1)	CU+2(+1)	A
24.70	CDT4(+1)	CU+2(+1) H +1(+1)	A
18.50	CDT4(+1)	FE+2(+1)	A
21.00	CDT4(+1)	FE+2(+1) H +1(+1)	A
28.00	CDT4(+1)	FE+3(+1)	A
28.50	CDT4(+1)	FE+3(+1) H +1(+1)	A
35.00	CDT4(+1)	FE+3(+1) OH-1(+1)	A
19.10	CDT4(+1)	PB+2(+1)	A
23.00	CDT4(+1)	PB+2(+1) H +1(+1)	A
10.90	CDT4(+1)	MG+2(+1)	A
16.40	CDT4(+1)	MN+2(+1)	A
19.50	CDT4(+1)	MN+2(+1) H +1(+1)	A
19.90	CDT4(+1)	NI+2(+1)	A
22.30	CDT4(+1)	NI+2(+1) H +1(+1)	A
18.60	CDT4(+1)	ZN+2(+1)	A
21.80	CDT4(+1)	ZN+2(+1) H +1(+1)	A
9.40	EDD2(+1)	H +1(+1)	A
16.00	EDD2(+1)	H +1(+2)	A
18.00	EDD2(+1)	H +1(+3)	A
19.50	EDD2(+1)	H +1(+4)	A
4.00	EDD2(+1)	CA+2(+1)	G
16.00	EDD2(+1)	CU+2(+1)	A
9.00	EDD2(+1)	FE+2(+1)	G
19.50	EDD2(+1)	FE+3(+1)	G
27.00	EDD2(+1)	FE+3(+1) OH-1(+1)	G
29.50	EDD2(+1)	FE+3(+1) OH-1(+2)	G

11.50	EDD2(+1)	PB+2(+1)		G
3.90	EDD2(+1)	MG+2(+1)		A
6.90	EDD2(+1)	MN+2(+1)		A
13.40	EDD2(+1)	NI+2(+1)		A
11.00	EDD2(+1)	ZN+2(+1)		A
9.30	EGT4(+1)	H +1(+1)		A
17.90	EGT4(+1)	H +1(+2)		A
20.50	EGT4(+1)	H +1(+3)		A
22.50	EGT4(+1)	H +1(+4)		A
10.30	EGT4(+1)	CA+2(+1)		A
28.00	EGT4(+1)	FE+3(+1)	OH-1(+1)	A
17.30	EGT4(+1)	CU+2(+1)		A
21.50	EGT4(+1)	CU+2(+1)	H +1(+1)	A
11.50	EGT4(+1)	FE+2(+1)		A
16.00	EGT4(+1)	FE+2(+1)	H +1(+1)	A
20.00	EGT4(+1)	FE+3(+1)		A
28.50	EGT4(+1)	FE+3(+1)	OH-1(+1)	G
14.30	EGT4(+1)	PB+2(+1)		A
19.50	EGT4(+1)	PB+2(+1)	H +1(+1)	A
5.00	EGT4(+1)	MG+2(+1)		A
12.60	EGT4(+1)	MG+2(+1)	H +1(+1)	A
12.00	EGT4(+1)	MN+2(+1)		A
16.00	EGT4(+1)	MN+2(+1)	H +1(+1)	A
13.30	EGT4(+1)	NI+2(+1)		A
18.40	EGT4(+1)	NI+2(+1)	H +1(+1)	A
12.40	EGT4(+1)	ZN+2(+1)		A
17.30	EGT4(+1)	ZN+2(+1)	H +1(+1)	A
11.55	EHP4(+1)	H +1(+1)		A
21.55	EHP4(+1)	H +1(+2)		A
30.10	EHP4(+1)	H +1(+3)		A
36.30	EHP4(+1)	H +1(+4)		A
7.00	EHP4(+1)	CA+2(+1)		A
16.00	EHP4(+1)	CA+2(+1)	H +1(+1)	A
23.00	EHP4(+1)	CA+2(+1)	H +1(+2)	A
24.00	EHP4(+1)	CU+2(+1)		A
32.00	EHP4(+1)	CU+2(+1)	H +1(+1)	A
37.00	EHP4(+1)	CU+2(+1)	H +1(+2)	A
33.50	EHP4(+1)	FE+3(+1)		A
19.00	EHP4(+1)	PB+2(+1)		G
7.70	EHP4(+1)	MG+2(+1)		A
16.50	EHP4(+1)	MG+2(+1)	H +1(+1)	A
24.00	EHP4(+1)	MG+2(+1)	H +1(+2)	A
19.50	EHP4(+1)	NI+2(+1)		A
27.00	EHP4(+1)	NI+2(+1)	H +1(+1)	A
32.80	EHP4(+1)	NI+2(+1)	H +1(+2)	A
16.50	EHP4(+1)	ZN+2(+1)		A
24.00	EHP4(+1)	ZN+2(+1)	H +1(+1)	A
30.50	EHP4(+1)	ZN+2(+1)	H +1(+2)	A
9.30	HDT3(+1)	H +1(+1)		A
14.70	HDT3(+1)	H +1(+2)		A
17.30	HDT3(+1)	H +1(+3)		A
8.00	HDT3(+1)	CA+2(+1)		A
11.00	HDT3(+1)	CA+2(+1)	H +1(+1)	A
17.20	HDT3(+1)	CU+2(+1)		A
19.00	HDT3(+1)	CU+2(+1)	H +1(+1)	A

12.00	HDT3(+1)	FE+2(+1)	A
14.50	HDT3(+1)	FE+2(+1) H +1(+1)	A
16.50	HDT3(+1)	FE+2(+1) OH-1(+1)	A
19.70	HDT3(+1)	FE+3(+1)	A
15.50	HDT3(+1)	FE+3(+1) H +1(-1)	A
6.50	HDT3(+1)	FE+3(+1) H +1(-2)	A
30.00	HDT3(+2)	FE+3(+2) H +1(-2)	A
15.00	HDT3(+1)	PB+2(+1)	A
11.50	HDT3(+1)	PB+2(+1) H +1(+1)	A
6.50	HDT3(+1)	HG+2(+1)	A
10.70	HDT3(+1)	HG+2(+1) H +1(+1)	A
10.50	HDT3(+1)	HN+2(+1)	A
11.00	HDT3(+1)	MN+2(+1) H +1(+1)	G
16.80	HDT3(+1)	NI+2(+1)	A
19.20	HDT3(+1)	NI+2(+1) H +1(+1)	A
14.20	HDT3(+1)	ZN+2(+1)	A
16.20	HDT3(+1)	ZN+2(+1) H +1(+1)	G
9.30	ODT4(+1)	H +1(+1)	A
17.90	ODT4(+1)	H +1(+2)	A
20.50	ODT4(+1)	H +1(+3)	A
22.20	ODT4(+1)	H +1(+4)	A
9.80	ODT4(+1)	CA+2(+1)	A
14.00	ODT4(+1)	CA+2(+1) H +1(+1)	A
17.80	ODT4(+1)	CU+2(+1)	A
21.50	ODT4(+1)	CU+2(+1) H +1(+1)	A
14.00	ODT4(+1)	FE+2(+1)	A
17.10	ODT4(+1)	FE+2(+1) H +1(+1)	A
24.20	ODT4(+1)	FE+3(+1)	A
30.00	ODT4(+1)	FE+3(+1) OH-1(+1)	G
14.70	ODT4(+1)	PB+2(+1)	A
18.20	ODT4(+1)	PB+2(+1) H +1(+1)	A
8.30	ODT4(+1)	HG+2(+1)	A
13.00	ODT4(+1)	MG+2(+1) H +1(+1)	A
13.40	ODT4(+1)	MN+2(+1)	A
16.50	ODT4(+1)	MN+2(+1) H +1(+1)	G
15.10	ODT4(+1)	NI+2(+1)	A
18.00	ODT4(+1)	NI+2(+1) H +1(+1)	A
14.80	ODT4(+1)	ZN+2(+1)	A
17.80	ODT4(+1)	ZN+2(+1) H +1(+1)	A
9.50	NTA3(+1)	H +1(+1)	A
12.00	NTA3(+1)	H +1(+2)	A
14.00	NTA3(+1)	H +1(+3)	A
15.00	NTA3(+1)	H +1(+4)	A
6.20	NTA3(+1)	CA+2(+1)	A
8.50	NTA3(+2)	CA+2(+1)	A
13.00	NTA3(+1)	CU+2(+1)	A
17.00	NTA3(+2)	CU+2(+1)	A
17.00	NTA3(+1)	CU+2(+1) OH-1(+1)	A
8.00	NTA3(+1)	FE+2(+1)	A
12.50	NTA3(+2)	FE+2(+1)	A
9.60	NTA3(+1)	FE+2(+1) H +1(+1)	A
-3.00	NTA3(+1)	FE+2(+1) H +1(-1)	A
15.50	NTA3(+1)	FE+3(+1)	A
24.00	NTA3(+2)	FE+3(+1)	A
11.00	NTA3(+1)	FE+3(+1) H +1(-1)	A

3.00	NTA3(+1)	FE+3(+1) H +1(-2)	A
25.00	NTA3(+2)	FE+3(+2) H +1(-2)	A
11.00	NTA3(+1)	PB+2(+1)	A
14.50	NTA3(+2)	PB+2(+1)	G
1.00	NTA3(+1)	PB+2(+1) H +1(-1)	G
5.20	NTA3(+1)	MG+2(+1)	A
7.00	NTA3(+2)	MG+2(+1)	G
7.20	NTA3(+1)	MN+2(+1)	A
10.20	NTA3(+2)	MN+2(+1)	A
11.30	NTA3(+1)	NI+2(+1)	A
15.80	NTA3(+2)	NI+2(+1)	A
-1.00	NTA3(+1)	NI+2(+1) H +1(-1)	A
10.30	NTA3(+1)	ZN+2(+1)	A
13.50	NTA3(+2)	ZN+2(+1)	A
0.00	NTA3(+1)	ZN+2(+1) H +1(-1)	A
4.4	ACT1(+1)	H +1(+1)	A
1.24	ACT1(+1)	CA+2(+1)	A
1.9	ACT1(+2)	CA+2(+1)	G
1.51	ACT1(+1)	CU+2(+1)	A
2.31	ACT1(+2)	CU+2(+1)	A
1.21	ACT1(+1)	MG+2(+1)	A
1.8	ACT1(+2)	MG+2(+1)	G
1.22	ACT1(+1)	ZN+2(+1)	E
2.0	ACT1(+2)	ZN+2(+1)	E
4.6	ANT1(+1)	H +1(+1)	A
6.4	ANT1(+1)	H +1(+2)	E
0.67	ANT1(+1)	CA+2(+1)	E
4.1	ANT1(+1)	CU+2(+1)	A
7.3	ANT1(+2)	CU+2(+1)	G
0.72	ANT1(+1)	MG+2(+1)	E
2.5	ANT1(+1)	ZN+2(+1)	A
4.5	ANT1(+2)	ZN+2(+1)	G
3.44	ASA1(+1)	H +1(+1)	M
2.94	ASA1(+1)	CA+2(+1)	M
4.7	ASA1(+2)	CA+2(+1)	G
1.26	ASA1(+1)	CU+2(+1)	M
3.03	ASA1(+2)	CU+2(+1)	M
2.29	ASA1(+1)	MG+2(+1)	M
4.0	ASA1(+2)	MG+2(+1)	G
2.5	ASA1(+1)	ZN+2(+1)	G
4.3	ASA1(+2)	ZN+2(+1)	G
4.8	CIQ1(+1)	H +1(+1)	E
7.6	CIQ1(+1)	CU+2(+1)	G
14.35	CIQ1(+2)	CU+2(+1)	G
3.9	CIQ1(+1)	ZN+2(+1)	G
8.0	CIQ1(+2)	ZN+2(+1)	G
6.2	FEN0(+1)	H +1(+1)	G
6.0	FEN0(+1)	CU+2(+1)	G
11.0	FEN0(+2)	CU+2(+1)	G
3.0	FEN0(+1)	ZN+2(+1)	G
5.8	FEN0(+2)	ZN+2(+1)	G
8.796	DPA1(+1)	H +1(+1)	M
14.65	DPA1(+1)	H +1(+2)	M
16.62	DPA1(+1)	H +1(+3)	M
10.587	DPA1(+1)	CU+2(+1)	M

18.775	DPA1(+2)	CU+2(+1)	M
14.973	DPA1(+1)	CU+2(+1) H +1(+1)	M
24.088	DPA1(+2)	CU+2(+1) H +1(+1)	M
28.2	DPA1(+2)	CU+2(+1) H +1(+2)	M
5.20	DPA1(+1)	FE+2(+1)	G
9.50	DPA1(+2)	FE+2(+1)	G
4.50	DPA1(+1)	FE+3(+1)	G
8.00	DPA1(+2)	FE+3(+1)	G
8.148	DPA1(+1)	NI+2(+1)	M
14.966	DPA1(+2)	NI+2(+1)	M
13.224	DPA1(+1)	NI+2(+1) H +1(+1)	M
20.49	DPA1(+2)	NI+2(+1) H +1(+1)	M
6.38	DPA1(+1)	ZN+2(+1)	M
11.67	DPA1(+2)	ZN+2(+1)	M
12.44	DPA1(+1)	ZN+2(+1) H +1(+1)	M
17.97	DPA1(+2)	ZN+2(+1) H +1(+1)	M
3.24	DPA1(+1)	MN+2(+1)	G
6.16	DPA1(+2)	MN+2(+1)	G
9.6	DPA1(+1)	MN+2(+1) H +1(+1)	G
5.96	DPA1(+1)	PB+2(+1)	G
9.00	DPA1(+2)	PB+2(+1)	G
6.1	THP1(+1)	H +1(+1)	G
7.6	THP1(+1)	H +1(+2)	G
6.0	THP1(+1)	CU+2(+1)	G
11.2	THP1(+2)	CU+2(+1)	G
3.1	THP1(+1)	ZN+2(+1)	G
5.6	THP1(+2)	ZN+2(+1)	G
3.7	THP1(+1)	NI+2(+1)	G
6.8	THP1(+2)	NI+2(+1)	G
8.2	THP1(+3)	NI+2(+1)	G
7.5	SGL2(+1)	H +1(+1)	G
8.6	SGL2(+1)	H +1(+2)	G
6.9	SGL2(+1)	CU+2(+1)	G
12.7	SGL2(+2)	CU+2(+1)	G
4.2	SGL2(+1)	NI+2(+1)	G
7.6	SGL2(+2)	NI+2(+1)	G
3.7	SGL2(+1)	ZN+2(+1)	G
7.9	SGL2(+2)	ZN+2(+1)	G
3.9	ATH1(+1)	H +1(+1)	G
1.8	ATH1(+1)	CU+2(+1)	G
1.0	ATH1(+1)	NI+2(+1)	G
1.5	ATH1(+1)	ZN+2(+1)	G

APPENDIX A3

Instructions and FORTRAN listings of the computer programs MIX and ECCLES

ECCLES AND MIX

DATA PREPARATION FOR COMPUTER SIMULATION PROCEDURES USING THE ECCLES AND MIX COMPUTER PROGRAMS .

This document describes how the input data for the ECCLES and MIX computer programs in the Bio-inorganic Chemistry computer library held by the South West Universities Computer Network should be punched onto data cards. It refers only to the simulation of metal ion-ligand equilibria in aqueous solutions where the free hydrogen ion concentration and all the formation constants of the reactions are known. A separate document, entitled "The MIXECC Macro', gives details of the Job Control commands required to call and execute the programs.

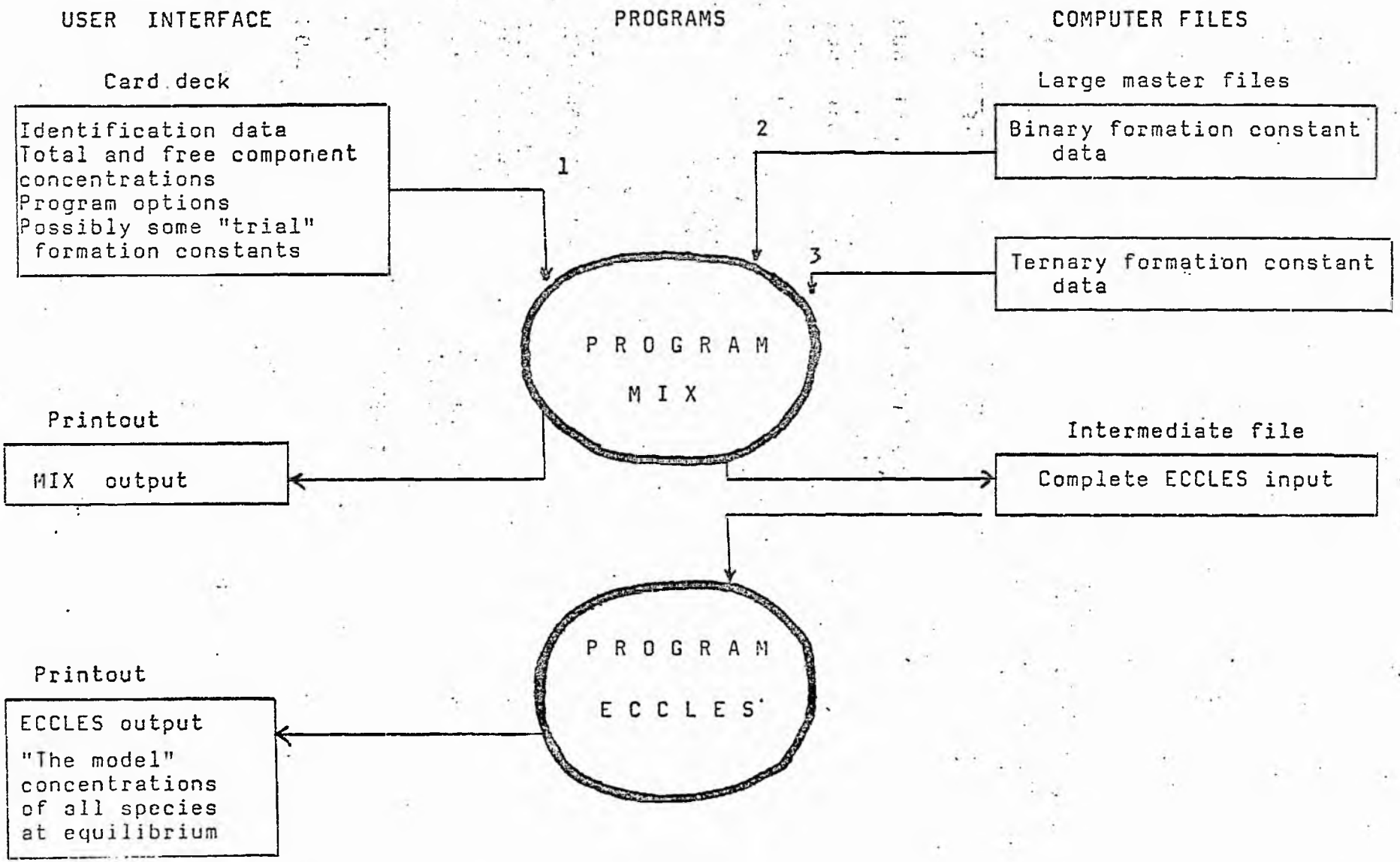
Introduction

The simulation of the equilibrium distribution of metal ions, ligands and their complexes involves the computation of the concentrations of all the species which co-exist in a defined solution. This requires a knowledge of the formation constants of each equilibrium reaction and of the total or free concentrations of each component in the mixture.

In the present context, these calculations are performed by program ECCLES. The name stands for Evaluation of Constituent Concentrations in Large Equilibrium Systems. The program was specifically designed to treat cases in which there were many components and very many (i.e. thousands) of complex species. Several of its facilities reflect this. For example, ECCLES identifies each component by a user-designated, four-character symbol so that restrictions on the number or type of component are minimised. Strings of these symbols interspersed with indications of the appropriate stoichiometry are used to define complex species. (This is described under Stage 8 of these instructions.) Users should be aware that in some regards smaller systems are processed somewhat inefficiently by ECCLES. Nevertheless, the program has several features not offered by other simulation outlines, so its use in the study of systems with only two or three components is often justified.

The most obvious of these features concerns utilization of the large formation constant database originally built up for the simulation of metal ion-ligand equilibria in blood plasma. Well over 2000 values have been critically selected from the literature. Users can access this database by implementing the MIXECC macro. This procedure first invokes program MIX to prepare input data for program ECCLES as shown in the Figure. Once the user has defined the system in terms of its components (using the symbols given in the instructions on the MIXECC macro) and their respective concentrations, program MIX generates the intermediate data file by performing three main tasks. These are (i) the selection of the applicable binary formation constants for the system in question from the database, (ii) the calculation of formation constant estimates for all ternary complex species that may be assumed to exist in the solution on the basis of the chosen binary formation constants and (iii) the substitution, adjustment or extension of these ternary formation constant estimates in respect of those for which experimental data has been collected.

Much of the input data for program MIX is prepared in exactly the same way as that for program ECCLES. Accordingly, it is convenient to describe the format of this data for both programs together. Note, however, that a number of the following instructions are simply transferred through program MIX to program ECCLES.


USER INTERFACE
PROGRAMS
COMPUTER FILES
Card deck
Identification data
Total and free component concentrations
Program options
Possibly some "trial" formation constants
Large master files
Binary formation constant data
Ternary formation constant data
1
2
3
PROGRAM MIX
Printout
MIX output
Intermediate file
Complete ECCLES input
PROGRAM ECCLES
Printout
ECCLES output
"The model" concentrations of all species at equilibrium

Stage 1: Identification

Three cards are required with alpha-numeric information for identification purposes. This is usually the title of the simulation, the investigator's name and the date.
(A failure in program MIX which warrants a termination of the simulation run is signalled to program ECCLES by over-writing the user's title with the phrase 'ABORT THIS RUN'. Accordingly, the word 'ABORT' should not appear as part of the title specified by the user on the first card image.)

FORMAT (20 A4)
FORMAT (10 A4)
FORMAT (10 A4)

Stage 2: The component monitor

One card is required to specify which, if any, of the components is to be specially monitored by the program. This means that after the equilibrium distribution has been calculated, all the complex species of the component in question are sorted into order of highest concentration so that a list of the topmost 35-40 can be printed.

This device is usually necessary only when there are many components in the equilibrium mixture. It then becomes tedious to sort through the usual output of all complex species looking for the most important of those which include a component of interest.

If no component monitor is desired, punch 'FALSE' in columns 1-5. Otherwise, punch the logical indicator 'TRUE ' followed by a list of symbols for up to 15 selected components. Monitored components must appear in at least three complex species. The word 'ALL' in columns 7-9 inclusive indicates that every component in the system is to be monitored. The word 'PUNCH' in columns 6-10 indicates that each monitor output list is to be sent to the card punch (device=7).

FORMAT (L5, 1X, 14 (A4, 1X), A4)

Stage 3: The component concentration scan

One card is required to specify which, if any, of the component concentrations is to be scanned. This means that the equilibrium distribution is repetitively calculated, first using the component concentration as specified under Stages 5 or 6 below and then at various increased values.

If no scan is desired, punch 'FALSE' in columns 1-5. Otherwise, punch the logical indicator 'TRUE ' followed by the component symbol in columns 7-10 inclusive. The factor or increment by which the component concentration is to be increased must be given in columns 11-20 and the maximum concentration of the component (after reaching which the scan is to be terminated) must appear in columns 21-30.

For example:

TRUE WXYZ 10.0 0.001

Component concentrations can be raised additively or in a multiplicative fashion: if the number in columns 11-20 is less than or equal to 1.00 it is taken to be an additive increment whereas otherwise it is taken as a multiplicative factor.

It is possible to scan the concentrations of up to ten other components simultaneously with that of the component stipulated in columns 7-10. This is done by listing the symbols of these other components from column 32 onwards. The same increment or factor will then be applied to the concentrations of those components.

FORMAT (L5, 1X, A4, 2G10.4, 10(1X, A4))

Stage 4: The program options

The following card images are optional but if included they must appear in the order shown below. Each string begins in column 1.

SUPPRESS OUTPUT

TEST PROGRAM

OMIT INPUT CHECKS

SWITCH DATA FILES

(a) The option to supress output.
This option is invoked when a lengthy print-out of all the complex species concentrations is not required. Most often this will occur when the necessary information is obtained through the component monitor (see Stage 2).

(b) The program test option.
This option terminates the program prematurely. It is occasionally useful as a means of checking an input deck but most often employed when ECCLES is being loaded onto a computer system for the first time. By itself, the 'TEST PROGRAM' command sets the iteration limit to zero. However, if the command is executed in conjunction with a component concentration scan card (under Stage 3) with 'TRUE' in columns 1-4) and 'TEST' in columns 7-10, the iteration limit will be set to the value given in columns 11-20. This value must be a real number between 1.0 and 500.0.

(c) The option to omit the input checking procedures.
Program ECCLES performs a large variety of checks whilst the input data is being loaded. The most expensive of these (in terms of computer time) involves a search for duplication amongst the list of complex species. This and a few other checking procedures can be bypassed by including the 'OMIT' option.

(d) The option to switch data files.
It is sometimes convenient to have program MIX prepare an intermediate data file which contains only the formation constant list. In other words, the data for ECCLES is submitted in two parts: (i) the input described in Stages 1-6 of this document on computer cards and (ii) that detailed in Stage 8, in the intermediate computer file by itself. This situation arises most commonly when a series of models are all based on the same components but utilize assorted concentration conditions. As the formation constant set is unchanged from one model to another it is uneconomic to generate it again and again using program MIX. Rather, the list is prepared on the first occasion only and then accessed by ECCLES whenever it is subsequently required. This may be accomplished by including the 'SWITCH' option in the data check. Program MIX executes the instruction by obliterating from the intermediate file all data required by ECCLES which precedes the list of formation constants. Program ECCLES, on the other hand, switches its internal logical device number for input so that the formation constants can be read in from a separate computer file (with device = 9) as opposed to that containing the initial input data (device = 5).

FORMAT (A4)

Stage 5: Total concentration input

This stage of the input is initiated by a card reading 'TOTAL CONCENTRATIONS' and terminated by a card reading 'END'. Both images begin in column 1.

FORMAT (A4)

Between these delimiters are placed the cards defining the symbols and concentrations of the components in the equilibrium solution whose total concentrations are known. The components, one per card image, may appear in any order.

The four-character component symbol specified by the user or as taken from the instructions on the MIXECC macro is placed in columns 1-4. This is followed by the total concentration of the component and if available, by an estimate of its free concentration. The free concentration estimate is mandatory for program MIX.

FORMAT (A4, 1X, 2G10.4).

Stage 6: Free concentration input

This stage of the input is initiated by a card reading 'FREE CONCENTRATIONS' and terminated by a card reading 'END'. Both images begin in column 1.

FORMAT (A4)

Between these delinities are placed the cards defining the symbols and concentrations of the components in the equilibrium solution whose free concentrations are known. The components, one per card image, may appear in any order.

The four-character component symbol specified by the user or as taken from the instructions on the MIXECC Macro is placed in columns 1-4. This is followed by the free concentration of the component and if available by an estimate of its total concentration. The total concentration estimate is mandatory for program MIX.

FORMAT (A4, 1X, 2G10.4)

Note: Although ECCLES does not require any free concentrations it would be most unusual for a user not to provide one in respect of the hydrogen ion. This is because ECCLES cannot process total hydrogen ion concentrations. The restriction arises from the choice of iterative formulae, these being unable to accommodate negative total concentrations. Thus, the hydroxide ion must be regarded as a separate component whose free concentration is supplied by the user from a knowledge of the dissociation constant for water. The latter parameter must not be included in the formation constant list (described under Stage 8). Its value is calculated by ECCLES as the product of the two free ion concentrations provided by the user at this stage. This is necessary when, for example, the free hydrogen ion concentration is to be scanned and the free hydroxide ion concentration must be adjusted accordingly. Program ECCLES will accept complex species defined in terms of a negative hydrogen subscript but these are immediately transformed by the program into the corresponding hydroxo-complexes (with appropriately altered formation constant values).

Stage 7: Selector options for program MIX

The selector option is only recognised by program MIX. It is used to exclude species in the formation constant database from the current model. In other words, the species in question are not transferred to the intermediate computer file which is to be used for ECCLES input. The option is effected by the command 'BLOCK' in columns 1-5 followed by a list of four-character component symbols which begin in column 7 and are separated from each other by a blank space.

For example:

```
BLOCK  WXYZ  PQRS  ABCD
```

Program MIX will omit any species in the masterfiles which contains all of the listed components.

The 'BLOCK' command may be repeated as often as required. Redundant combinations such as the one shown below should be avoided.

```
BLOCK  PQRS  WXYZ  ABCD
BLOCK  PQRS  ABCD
```

The 'BLOCK' option is used most often to include a new set of formation constants in a model without having to alter the masterfiles. These "trial" constants are included in the card deck as described under Stage 8. Note that users must include 'BLOCK' commands to ensure that duplication of these species from the masterfiles is impossible. In other words, each of the complex species entered via the card deck must be encompassed by the set of 'BLOCK' exclusion criteria. This remains the case even when they include components which are not part of the masterfile database.

```
FORMAT (A4, 2X, 10 (A4, 1X))
```

Stage 8: formation constant input

This stage of the input is initiated by a card reading 'SPECIES CONSTANTS'. The image begins in column 1.

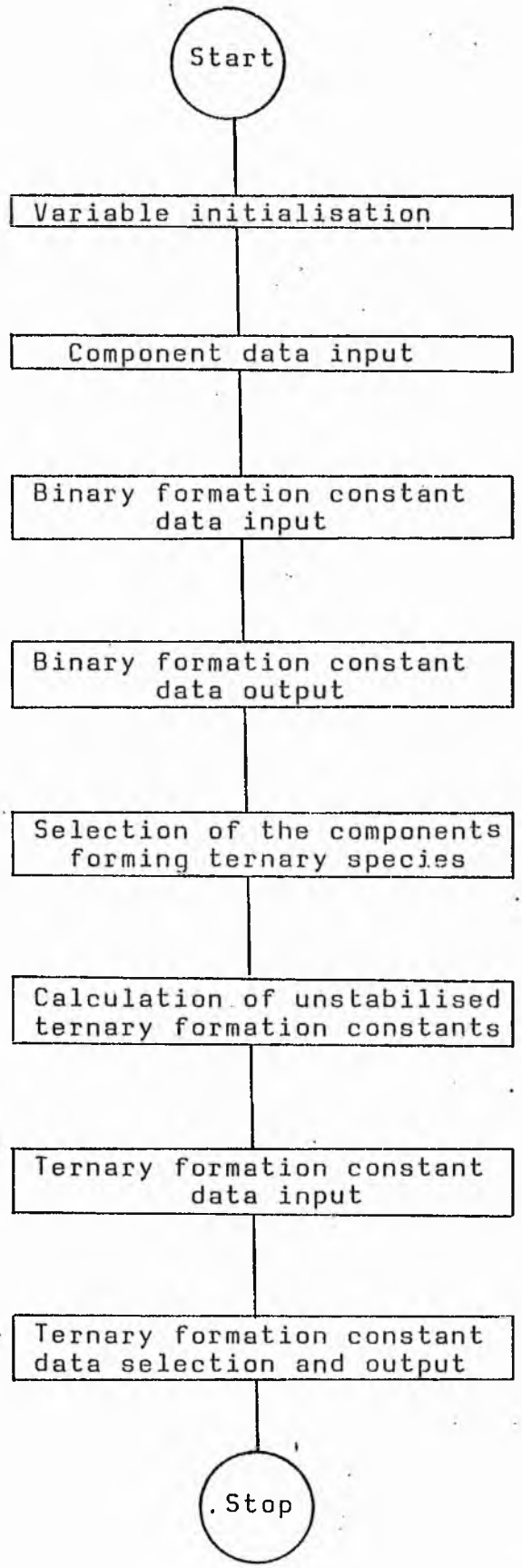
The cards following this each define the formation constant value and stoichiometry of a complex species. The formation constant is entered as a log value in columns 1-9. The complex is specified by an alphanumeric string beginning in column 11. The string comprises the various component symbols, each followed by its stoichiometric ratio as a signed integer in parentheses and separated from the next one by a blank space.

This is shown in the following examples:

```
16.957     WXYZ(+1) ABCD(+1)
22.322     WXYZ(+1) ABCD(+2)
 9.650     CU+2(+1) GLY1(+1) H +1(+1)
-2.2       CU+2(+1) GLY1(+1) H +1(-1)
FORMAT (G9.4, 1X, 5(A4, 1X, I2, 2X))
```

Program ECCLES is currently dimensioned to accept up to 5000 complex species. There is no equivalent limit for Program MIX but note the restrictions discussed under Stage 7 of this document.

FLOW DIAGRAM FOR PROGRAM MIX



```
C     PROGRAM MIX.
C
C     DEVELOPED AT THE UNIVERSITY OF CAPE TOWN DURING
                1975.
C
C     THIS PROGRAM PRODUCES, ON FILE, THE EQUILIBRIUM
                DATA REQUIRED AS INPUT
C     FOR PROGRAM ECCLES.
C
C     FOR A GIVEN SET OF MODEL COMPONENTS, IT SELECTS THE
                APPROPRIATE COMPLEX
C     SPECIES FROM A STANDARD FORMATION CONSTANT DATA
                FILE AND OUTPUTS THESE
C     AS WELL AS PROJECTED VALUES FOR ALL THE 1:1:1 MIXED
                LIGAND CONSTITUENTS
C     OF THE MIXTURE.   A USER SPECIFIED DEFAULT VALUE IS
                USED AS A
C     STABILISATION FACTOR UNLESS A MORE SPECIFIC VALUE
                CAN BE TAKEN DIRECTLY
C     OR CALCULATED FROM DATA SUPPLIED IN A FILE ON MIXED
                LIGAND COMPLEXES.
C     (THIS FILE MAY ALSO CONTAIN SPECIES TO BE OUTPUT
                WITHOUT ALTERATION).
C     SPECIES WHICH HAVE COMPONENTS THAT DO NOT APPEAR IN
                THE COMPONENT LIST
C     ARE IGNORED.   THIS FACILITATES MANIPULATION OF THE
                INPUT TO ECCLES.
C     THE PROGRAM IS WRITTEN IN FORTRAN IV.
C
C
C     SECTION ONE      STORAGE ALLOCATION AND FORMAT
                STATEMENTS.
C
C
C
      INTEGER  KEY(5,1500,2), NUM1(1500), NUM2(1500)
      INTEGER  DUP(116,2),  OMIT(1000,2)
C     BOTH OF THE ABOVE INTEGER SPECIFICATIONS COULD BE
                INTEGER*2
      INTEGER OUT, OUTF, NCOMP
      INTEGER  BUF(20), PCOMP(100), CHEK, PURGE(5,10)
      DIMENSION  CONST(1500), NCOMP(116), NDUM(10),
                LIT(10)
      DIMENSION  CONSTM(116), MCOMP(116), CONSTN(100),
                NCOMP(100)
      DIMENSION  CMIX(5000), STAB(5000), CBUF(116)
      DIMENSION  METARR(15), STBARR(15), XX(116),
                TREAL(116), XXM(116)
      REAL  LGMIX(5000), TREALM(116)
      LOGICAL  SWTCH, SUPRES, IPRT, ERCONC, BLOCK
      DATA  LIT/'    TOTAFREESPECH +1END SWITBLOC
                '/
C
C
C
```

```
      NS = 1500
      NX = 100
      NXX = 15
      NKEY = 5
      NPMAX = 100
      NDMAX = 110
      NZMAX = 1000
      NND = NKEY * 2
      NXP1 = NX + 1
      NSP1 = NS + 1
      NXXX = NX + NXX
      NMIX = (NX ** 2) / 2
C
C
C
10201 FORMAT(I4)
10301 FORMAT(20A4)
10303 FORMAT(A4,1X,2G10.4)
10330 FORMAT(A4,2X,10(A4,1X))
10401 FORMAT(G9.4,1X,5(A4,1X,I2,2X))
10501 FORMAT(A4,1X,G10.4)
20303 FORMAT(A4,1X,1P2E10.4,55(1H ))
20304 FORMAT('END',77(1H ))
20331 FORMAT('SPECIES CONSTANTS',63(1H ))
20401 FORMAT(F9.4,1X,5(A4,I3,2X),T80,A1)
20999 FORMAT('ABORT THIS RUN.   ERROR IN THE MIXED LIGAND
                  CONSTANT CALCU
     1LATIONS.    ',10(1H-))
30300 FORMAT(1H ,'MISSING INSTRUCTION IN DATA. PROGRAM
                  TERMINATED AFTER
     1CARD ',I3)
30201 FORMAT('1')
30301 FORMAT(' ',20A4)
30302 FORMAT(//'0','END OF COMPONENT DATA.
                  CARD IMAGES READ =',I3,///)
30303 FORMAT(' ',A4,1X,2(1PE12.4))
30304 FORMAT(' END')
30305 FORMAT(//'0','NO COMPONENT DATA OUTPUT TO THE
                  SEQUENTIAL FILE.')
30331 FORMAT(' ','SPECIES CONSTANTS')
30332 FORMAT(' ','BLOCK ',10(A4,1X))
30334 FORMAT('0','ERROR TERMINATION',/'0',
     1  'BLOCK OPTION SPECIFIES UNIDENTIFIABLE
                  COMPONENT',5X,A4,///)
30401 FORMAT(' ','OMIT....  ',1PE12.4,1X,5(A4,'(',I2,')',
                  1X))
30402 FORMAT(/'0','SPECIES',I6,'  ON CARD NUMBER',I5,' IS
                  IN ERROR',///)
30403 FORMAT(//'0','END OF BASIC STABILITY CONSTANT DATA.
                    CARD IMAGES
     1READ =',I5,5X,'TOTAL NUMBER OF SPECIES =',I5,////)
30410 FORMAT(' ','INSERT ....',5X,F9.4,1X,5(A4,'(',I2,')',
                  1X))
30416 FORMAT('0','NO. FREE HYDROGEN ION CONCENTRATION???')
```

```
30417 FORMAT('0','NPMAX EXCEEDED.   EXECUTION
                  TERMINATED.')
30418 FORMAT('0','ERROR TERMINATION',/'0',
     1  'LAST SPECIES NOT COVERED BY THE BLOCK
                  OPTION',///)
30494 FORMAT(///'0','THE FOLLOWING METAL IONS ARE
                  RECOGNISED.',/'0')
30495 FORMAT(' ',A4,1PE14.3)
30501 FORMAT('0',A4,' IS NOT IN THE COMPONENT LIST.',/'
                  PLEASE RETYPE.
     '    TYPE END TO EXIT.')
30502 FORMAT('0',10(1H*),5X,'CHECK THIS SPECIES',5X,3(A4,
                  '(',I2,')',2X))
30601 FORMAT('0','EXECUTION TERMINATED.
                  MIXED SPECIE ARRAYS TOO SMALL')
30602 FORMAT('0','OMIT THE PREDICTION FOR',5X,1PE10.4,5X,
                  A4,'( 1) ',
     1 A4,'( 2) H +1( 1)',4X,I6)
30603 FORMAT('0','COMBINE PREDICTIONS FOR IDENTICAL MIXED
                  SPECIES.',
     1 /' ',1PE10.4,5X,3(A4,'( 1) '),'H +1(
                  1)',5X,2(1PE10.4),3I6)
30604 FORMAT(' ',A4,5X,3(1PE15.4,5X))
30605 FORMAT(//'0','THE FOLLOWING COMPONENTS ARE USED FOR
                  THE ',A4,
     1' TERNARY CONSTANT CALCULATIONS:',///)
30606 FORMAT(//'0','TOTAL NUMBER =',I4,/' ','NUMBER OF
                  BARE LIGANDS =',
     1  I4,/' ','NUMBER WITH ONE PROTON =',I4,/////)
30607 FORMAT(//'0','METAL CONCENTRATION FACTORS:',
                  2(1PE15.4))
30731 FORMAT(' OUTPUT TO FILE ',10X,1PE10.4,1X,8(A4,'(',
                  I2,')',1X))
30741 FORMAT(' COMPONENT NOT IN LIST.')
30742 FORMAT('0','OMIT THE SPECIE ON CARD NUMBER',I4,//)
30745 FORMAT(///'0','ERROR ON CARD NUMBER',I4,//)
30746 FORMAT(' MODE CODE =',I4,//)
30747 FORMAT(///'0','ERROR TERMINATION.
                  FORMAT ERROR ON CARD',I5,///)
30748 FORMAT('0','STABILISATION FACTOR HAS ALREADY BEEN
                  SET.')
30749 FORMAT('0','MIXED SPECIES MUST HAVE  H +1
                  AS THE LAST COMPONENT')
30761 FORMAT(//'0','A BINARY CONSTANT FOR MIXED LIGAND
                  STABILITY CALCULA
     1TION IS MISSING',/' ','OR A NECCESSARY BINARY
                  SPECIES DOES NOT APP
     1EAR IN THE BASIC DATA FILE.',///)
30763 FORMAT('0','PREDICTION FOR ',8(A4,'(',I2,')',1X))
30764 FORMAT(' ',T40,4(1PE12.4))
30771 FORMAT(' ','ADJUST THE STAB. FACTOR ',10X,3(A4,'(
                  1)',1X),'H +1(',
     1  I2,')',4X,2(1PE10.4))
30781 FORMAT('0REPLACED SPECIE',10X,1PE10.4,1X,8(A4,'(',
                  I2,')',1X))
```

```
30801 FORMAT('0',I5,' CONSTANTS FOR THE MIXED LIGAND
                  COMPLEXES OF ',A4,
     1' HAVE BEEN CONSIDERED.',/' ',I5,' HAVE BEEN
                  SELECTED.',/' ',
     2'THE ELIMINATION FACTOR WAS',1PE14.4)
30802 FORMAT('0','NUMBER OF APPLICABLE CARDS IN THE
                  MIXED-DATA FILE =',
     1  I5,////)
30892 FORMAT(' ',I6,2I8,1PE15.4,5X,'OMIT  =
                  IDENTICAL TO SPECIES NUMBER
     1',I5)
30893 FORMAT(' ',I6,2I8,6(1PE15.4))
30899 FORMAT('0','IMPERFECT ELIMINATION.',I6,' COMPLEXES
                  SHOULD HAVE BEE
     1N SELECTED.',//)
30901 FORMAT(//'0',I5,' CONSTITUENTS FOR ECCLES!')
30902 FORMAT(//'0','END MIX.',/'1')
30903 FORMAT('0',I5,' SPECIES WERE SELECTED FROM A TOTAL
                  OF',I6,' THAT W
     1ERE CONSIDERED.')
30994 FORMAT('0','FREE CONCENTRATION GREATER THAN TOTAL!')
30995 FORMAT('0','SYMBOL ERROR IN METAL STABILISATION
                  FACTOR FILE')
30996 FORMAT('0','SYMBOL ERROR IN BASIC CONSTANT DATA
                  FILE.',5X,A4)
30997 FORMAT('0','SYMBOL ERROR IN MIXED CONSTANT DATA
                  FILE.',5X,A4)
30998 FORMAT('0','ERROR ON CARD NUMBER',I6,////)
30999 FORMAT(///'0','ABORT ECCLES RUN.')
C
C
C
C     SECTION TWO       INITIALISATION.
C
C
  200 IN = 5
      IN1 = 9
      IN2 = 3
      IN3 = 4
      IN8 = IN1
      JSTART = 1
      OUT = 6
      OUTF = 7
      N1 = NXP1
      N2 = NXX + 1
      N3 = NSP1
      NNN = 0
      NPURGE = 0
      SWTCH = .FALSE.
      NREJEK = 0
      WRITE(OUT,30201)
      SUPRES = .TRUE.
      IPRT = .FALSE.
      ERCONC = .FALSE.
      GO TO 300
```

```
  250 IPRT = .TRUE.
      SUPRES = .FALSE.
C
C
C
C     SECTION THREE.      COMPONENT DATA INPUT AND OUTPUT.
C
C
  300 READ(IN1,10301) IXA, IXB, NDUM, I, J, K, L, M, N,
                      NN, NM
      NNN = NNN + 1
      IF (NNN .GT. 15) WRITE(OUT,30300) NNN
      WRITE(OUT,30301) IXA, IXB, NDUM, I, J, K, L, M, N,
                      NN, NM
      IF(NNN.EQ.1.AND.IXA.EQ.LIT(3))  GO TO 250
      WRITE(OUTF,10301) IXA, IXB, NDUM, I, J, K, L, M, N,
                      NN, NM
      IF(IXA.EQ.LIT(7))  SWTCH = .TRUE.
      IF(IXA.NE.LIT(2))  GO TO 300
C
C
C
      DO 301 NI=1,N1
      READ(IN1,10303)  IXA, AXA, BXA
      NNN = NNN + 1
      IF (NNN .GT. 120) GO TO 3011
      IF(IXA.EQ.LIT(6))  GO TO 302
      WRITE(OUTF,20303)  IXA, AXA, BXA
      WRITE(OUT,30303)  IXA, AXA, BXA
      IF(BXA.LT.1.0E-28)  BXA = 1.0E-28
      TREAL(NI) = AXA
      XX(NI) = BXA
      IF(XX(NI).LE.0.0000)  ERCONC = .TRUE.
  301 NCOMP(NI) = IXA
      STOP 301
 3011 WRITE(OUT,30300) NNN
      GO TO 9999
  302 NI = NI - 1
      WRITE(OUTF,20304)
      WRITE(OUT,30304)
      READ(IN1,10301) IXA, IXB, NDUM, I, J, K, L, M, N,
                      NN, NM
      NNN = NNN + 1
      WRITE(OUTF,10301) IXA, IXB, NDUM, I, J, K, L, M, N,
                      NN, NM
      WRITE(OUT,30301) IXA, IXB, NDUM, I, J, K, L, M, N,
                      NN, NM
      IF(IXA.NE.LIT(3))  GO TO 9998
C
C
C
      N=NI
      DO 303 NII=1,N2
      READ(IN1,10303)  IXA, AXA, BXA
      NNN = NNN + 1
```

```
      IF (NNN .GT. 150) GO TO 3011
      IF(IXA.EQ.LIT(6))  GO TO 304
      WRITE(OUTF,20303)  IXA, AXA, BXA
      WRITE(OUT,30303)  IXA, AXA, BXA
      IF(BXA.LT.1.0E-20)  BXA = 1.0E-20
      N = N + 1
      TREAL(N) = BXA
      XX(N) = AXA
      IF(TREAL(N).LT.XX(N))  ERCONC = .TRUE.
      IF(TREAL(N).LE.0.0000)  ERCONC = .TRUE.
  303 NCOMP(N) = IXA
      STOP 303
  304 NII = NII - 1
      WRITE(OUTF,20304)
      WRITE(OUT,30304)
      NIII = NI + NII
      IF(SWTCH)  REWIND  OUTF
      IF(SWTCH)  WRITE(OUT,30305)
      WRITE(OUT,30302) NNN
C
C     BLOCK OPTION TO IGNORE SPECIES IN MASTER FILE.
C
  330 READ(IN1,10330,END=331)  IXA, NDUM
      IF(IXA.NE.LIT(4))  GO TO 332
  331 WRITE(OUTF,20331)
      WRITE(OUT,30331)
      SWTCH = .FALSE.
      GO TO 350
  332 IF(IXA.NE.LIT(8))  GO TO 3011
      WRITE(OUT,30332)  NDUM
      NPURGE = NPURGE + 1
      IF(NPURGE.GT.NND)  STOP 332
      DO 336 N=1,NKEY
      IF(NDUM(N).EQ.LIT(1))  GO TO 335
      DO 334 M=1,NIII
      IF(NDUM(N).EQ.NCOMP(M))  GO TO 336
  334 CONTINUE
      WRITE(OUT,30334)  NDUM(N)
      GO TO 9999
  335 IF(N.EQ.1)  STOP 335
  336 PURGE(N,NPURGE)  =  NDUM(N)
      GO TO 330
C
C      EXPAND LIST OF PERMITTED SYMBOLS.
C
  350 NNN = 0
      NP = 0
      DO 352 I=1,400
      READ(IN2,10301)    IXA
      NNN = NNN + 1
      IF(IXA.EQ.LIT(6))  GO TO 400
      DO 351 M=1,NIII
      IF(IXA.EQ.NCOMP(M))  GO TO 352
  351 CONTINUE
      IF(NCOMP(NIII).EQ.LIT(6))  GO TO 400
```

```
      NP = NP + 1
      IF(NP.GT.NPMAX)  GO TO 416
      PCOMP(NP) = IXA
  352 CONTINUE
C
C
C
C     SECTION FOUR.    BASIC STABILITY CONSTANT DATA
                    INPUT AND OUTPUT.
C
C
  400 DO 412 NJ=1,N3
  401 READ(IN8,10401,END=4410,ERR=413) CONST(NJ), NDUM
      NNN = NNN + 1
      IF(NPURGE.GT.0)  SWTCH = BLOCK(NDUM,PURGE,NND,NKEY,
                    NPURGE,LIT(1))
      IF(SWTCH.AND.IN8.EQ.IN2)  GO TO 401
      NUM1(NJ) = 0
      NUM2(NJ) = 0
      NPM = 1
      DO 402 N=1,NKEY
      KEY(N,NJ,1) = 0
  402 KEY(N,NJ,2) = 0
      DO 407 N=1,NND,2
      IF(NDUM(N).EQ.LIT(1))  GO TO 408
      IF(NDUM(N+1).EQ.0)  GO TO 413
  403 DO 404 M=NPM,NIII
      IF(NDUM(N).EQ.NCOMP(M)) GO TO 405
  404 CONTINUE
      IF(NPM.EQ.1)  ND = N
      IF(NPM.EQ.1)  NUM1(NJ) = 0
      IF(NPM.EQ.1)  NUM2(NJ) = 0
      IF(NPM.EQ.1) GO TO 409
      NPM = 1
      GO TO 403
  405 NPM = M
      IF(M.GT.NI) GO TO 406
      L = NUM1(NJ) + 1
      NUM1(NJ) = L
      KEY(L,NJ,1) = M
      GO TO 407
  406 L = NKEY - NUM2(NJ)
      NUM2(NJ) = NUM2(NJ) + 1
      KEY(L,NJ,1) = M - NI
  407 KEY(L,NJ,2) = NDUM(N+1)
  408 IF(NUM1(NJ)+NUM2(NJ).LE.1) GO TO 413
  409 DO 410 N=2,NND,2
      IF(NDUM(N).EQ.0) GO TO 411
  410 CONTINUE
      N = NND + 2
  411 NN = N - 2
      IXA = NUM1(NJ) + NUM2(NJ)
      IF(IXA.GT.1)  GO TO 4411
      IF(IXA.EQ.1)  GO TO 413
      IF(IPRT) WRITE(OUT,30401) CONST(NJ),(NDUM(N),N=1,NN)
```

```
      IF(NUM2(NJ).EQ.NN/2)  GO TO 401
      IF(CHEK(NDUM(ND),PCOMP,NP).EQ.-1)  GO TO 9996
      GO TO 401
C     ************************************************************
                  ************
 4410 IF(IN8.EQ.IN2)  GO TO 414
      IN8 = IN2
      JSTART = NJ
      NNN = NNN - NJ + 1
      GO TO 401
C      ***********************************************************
                  ********
 4411 IF(IN8.EQ.IN1)     WRITE(OUT,30410)
                  CONST(NJ), (NDUM(N), N=1,NN)
      IF(.NOT.SWTCH.AND.IN8.EQ.IN1)  GO TO 418
      NNX = NN + 1
      IF(NNX.GE.NND)  GO TO 412
      DO 4412 N=NNX,NND,2
      NDUM(N) = LIT(1)
 4412 NDUM(N+1) = 0
  412 WRITE(OUTF,20401) CONST(NJ), NDUM, LIT(1)
C      ***********************************************************
                  ********
      STOP
  413 WRITE(OUT,30402) NJ, NNN
      GO TO 9999
  414 NJ = NJ - 1
      WRITE(OUT,30403) NNN, NJ
      NTOT = NJ
      NMET = 0
      NHYDR = NI
      DO 415 II=1,NII
      NHYDR = NHYDR + 1
      IF(NCOMP(NHYDR).EQ.LIT(5))  GO TO 480
  415 CONTINUE
      WRITE(OUT,30416)
      GO TO 9999
  416 WRITE(OUT,30417)
      GO TO 9999
  418 WRITE(OUT,30418)
      GO TO 9999
C
C
C
C     SECTION FIVE       SELECTION OF THE MIXED COMPLEX
                  COMPONENTS.
C
C
  480 NNN = 1
      DO 481 N=1,NND,2
      NDUM(N) = LIT(1)
  481 NDUM(N+1) = 0
      READ(IN,10201,END=494,ERR=745)  NSECC
  490 NMET = NMET + 1
```

```
  491 READ(IN,10501,END=494,ERR=745)  METARR(NMET),
                  STBARR(NMET)
      IF(METARR(NMET).EQ.LIT(6))  GO TO 494
      IF(BLOCK(NDUM,PURGE,NND,NKEY,NPURGE,LIT(1)))
                  GO TO 491
      DO 492 I=1,NIII
      IF(NCOMP(I).EQ.METARR(NMET))  GO TO 493
  492 CONTINUE
      WRITE(OUT,30501)  METARR(NMET)
      IF(CHEK(METARR(NMET),PCOMP,NP).EQ.-1)  GO TO 9995
      GO TO 491
  493 METARR(NMET) = I
      GO TO 490
  494 NNN = NMET - 1
      IF(NNN.LE.0)  GO TO 900
      WRITE(OUT,30494)
      DO 495 I=1,NNN
      METAL = METARR(I)
  495 WRITE(OUT,30495)  NCOMP(METAL), STBARR(I)
      WRITE(OUT,30201)
      NSECC = NSECC - NJ - 2
C
C
C
  500 NMET = NMET - 1
      IF(NMET.LE.0)  GO TO 900
      METAL = METARR(NMET)
      STABF = STBARR(NMET)
C
C     IF(STABF.LT.0)   PRINT TERNARY CONSTANT DETAILS.
C
      MAXMIX = NSECC / NMET
      TOTLM = ALOG10(TREAL(METAL))
      FREEM = ALOG10(XX(METAL))
      NM = 0
      NH = 0
      NT = 0
      NNN = 0
      METALF = 0
      IF(METAL.GT.NI) METALF = METAL - NI
C
      DO 516 J=JSTART,NJ
      IF(METALF.GT.0) GO TO 504
      NL = NUM1(J)
      DO 503 L=1,NL
      IF(KEY(L,J,1).EQ.METAL) GO TO 506
  503 CONTINUE
      GO TO 516
  504 IF(NUM2(J).EQ.0) GO TO 516
      NL = NKEY - NUM2(J) + 1
      DO 505 L=NL,NKEY
      IF(KEY(L,J,1).EQ.METALF) GO TO 506
  505 CONTINUE
      GO TO 516
  506 IF(KEY(L,J,2).NE.1) GO TO 516
```

```
      KEY(L,J,2) = 2
      NN = NUM1(J)
      NL = NKEY - NUM2(J) + 1
      DO 507 M=1,NKEY
      IF(M.GT.NN.AND.M.LT.NL) GO TO 507
      IF(KEY(M,J,2).NE.2) GO TO 515
  507 CONTINUE
      NN = NN + NUM2(J)
      IF(NN.GT.2) GO TO 508
      NH = NH + 1
      CONSTM(NH) = CONST(J) / 2.0
      MCOMP(NH) = KEY(1,J,1)
      IF(L.EQ.1) MCOMP(NH) = KEY(NKEY,J,1) + NI
      GO TO 515
  508 IF(NN.GT.3) GO TO 515
      NL = NKEY - 1
      IF(KEY(NL,J,1).NE.II.AND.KEY(NKEY,J,1).NE.II) GO TO
                  514
      NH = NH + 1
      IF(NUM2(J).EQ.1) GO TO 513
      IF(L.GT.NUM1(J)) GO TO 512
      HCOMP(NH) = KEY(NL,J,1) + NI
      IF(KEY(NL,J,1).EQ.II) HCOMP(NH) = KEY(NKEY,J,1) + NI
  511 CONSTH(NH) = CONST(J) / 2.0
      GO TO 515
  512 HCOMP(NH) = KEY(1,J,1)
      GO TO 511
  513 HCOMP(NH) = KEY(1,J,1)
      IF(L.EQ.1) HCOMP(NH) = KEY(2,J,1)
      GO TO 511
  514 KEY(L,J,2) = 1
      NN = KEY(1,J,1)
      NL = KEY(NKEY,J,1) + NI
      IXA = 2
      IF(KEY(2,J,1).EQ.0)  IXA = NKEY - 1
      IXB = KEY(IXA,J,1)
      IF(KEY(2,J,1).EQ.0)  IXB = IXB + NI
      WRITE(OUT,30502)  NCOMP(NN), KEY(1,J,2),
                  NCOMP(IXB), KEY(IXA,J,2),
     1  NCOMP(NL), KEY(NKEY,J,2)
  515 KEY(L,J,2) = 1
  516 CONTINUE
C
C
C
C     SECTION SIX       CALCULATION OF THE UNSTABILISED
                  MIXED LIGAND CONSTANTS.
C
C
  600 NHH = NM + NH
      NHX = ((NHH ** 2) - NMH) / 2
      IF(NHX.GT.NMIX) GO TO 605
      DO 601 M=1,NHX
  601 STAB(M) = 550.0
      DO 602 I=1,NH
```

```
      N = NM + I
      NCOMP(N) = HCOMP(I)
  602 CONSTM(N) = CONSTH(I)
C
C
C
      J = 0
      DO 604 M=2,NMH
      N = M - 1
      DO 604 I=1,N
      J = J + 1
  604 CMIX(J) = CONSTM(M) + CONSTM(I)
      GO TO 610
  605 WRITE(OUT,30601)
      GO TO 9999
C
C
C
  610 WRITE(OUT,30605)  NCOMP(METAL)
      AXA = ALOG10(XX(NHYDR))
      DO 611 M=1,NMH
      NPM = HCOMP(M)
      MCOMP(M) = NCOMP(NPM)
      XXM(M) = ALOG10(XX(NPM))
      TREALM(M) = ALOG10(TREAL(NPM))
      IF(M.GT.NM)  XXM(M) = XXM(M) + AXA
  611 WRITE(OUT,30604)  MCOMP(M), CONSTM(M), XXM(M),
                        TREALM(M)
      WRITE(OUT,30607)  FREEM,  TOTLM
      WRITE(OUT,30606)  NMH, NM, NH
      DO 612 M=1,NH
      NPM = HCOMP(M)
  612 HCOMP(M) = NCOMP(NPM)
C
C
C
      ND = 0
      DO 623 I=1,NH
      DO 621 M=1,NM
      IF(HCOMP(I).EQ.MCOMP(M))  GO TO 622
  621 CONTINUE
      GO TO 623
  622 ND = ND + 1
      IF(ND.GT.NDMAX)  STOP 622
      DUP(ND,1) = I + NM
      DUP(ND,2) = M
  623 CONTINUE
      OMIT(1,1) = 0
      NZ = 0
      IF(ND.EQ.0)  GO TO 700
      DO 626 I=1,ND
      DO 626 L=I,ND
      M = DUP(I,1) - 1
      IF(I.NE.L)  GO TO 624
      M = (((M ** 2) - M) / 2) + DUP(I,2)
```

```
      J = 0
      N3 = DUP(L,2)
      XA = CMIX(M) + STABF
      IF(SUPRES)  GO TO 625
      WRITE(OUT,30602)  XA, NCOMP(METAL), NCOMP(N3), M
      GO TO 625
  624 J = (((M ** 2) - M) / 2) + DUP(L,2)
      M = DUP(L,1) - 1
      M = (((M ** 2) - M) / 2) + DUP(I,2)
      XA = CMIX(J)
      IF(CMIX(M).GT.XA)  XA = CMIX(M)
      AXA = CMIX(J) - CMIX(M)
      AXA = ABS(AXA)
      IF(AXA.LT.0.5)  XA = XA + 0.1
      IF(AXA.LT.0.1)  XA = XA + 0.075
      IF(AXA.LT.0.05)  XA = XA + 0.05
      XA = XA + STABF
      N1 = J - I
      N3 = DUP(I,1)
      N2 = DUP(L,2)
      IF(IPRT) WRITE(OUT,30603)  XA, NCOMP(METAL),
                  NCOMP(N3), NCOMP(N2),
     1  CMIX(J), CMIX(M), J, M, N1
      CMIX(J) = XA - STABF
  625 NZ = NZ + 1
      IF(NZ.GT.NZMAX)  STOP 625
      NMX = NMX - 1
      OMIT(NZ,2) = J
  626 OMIT(NZ,1) = M
C
C
C
C     SECTION SEVEN       INPUT, PROCESS AND OUTPUT MIXED
                  LIGAND DATA.
C
C
C
C     READ THE CARD;  TEST IF IT APPLIES TO THE CURRENT
                  METAL ION.
C
  700 KSW = 0
  701 NNN = NNN + 1
      READ(IN3,10401,END=800,ERR=746)  XA, NDUM
      IF(NDUM(1).EQ.LIT(1))  GO TO 770
      IF(NDUM(1).EQ.NCOMP(METAL))  GO TO 702
      DO 99701 I=1,NIII
      IF(NDUM(1).EQ.NCOMP(I))  GO TO 700
99701 CONTINUE
      N1 = 1
      IF(CHEK(NDUM(1),PCOMP,NP).EQ.-1)  GO TO 9997
      GO TO 700
C
C     DETERMINE WHETHER EACH COMPONENT APPEARS IN THE
                  CURRENT MODEL.
C
```

```
  702 IF(KSW.EQ.100)  KSW = 0
      NT = NT + 1
      I = 1
      DO 705 N=4,NND,2
      IF(NDUM(N).EQ.0)  GO TO 706
      N1 = N - 1
      N2 = I
  703 DO 704 I=N2,NIII
      IF(NCOMP(I).EQ.NDUM(N1))  GO TO 705
  704 CONTINUE
      IF(N2.EQ.1)  GO TO 740
      N2 = 1
      GO TO 703
  705 CONTINUE
      N = N + 2
  706 NN = N - 2
C
C     TEST FOR  'H +1'  POSITIONAL ERROR.
C
      IF(NDUM(1).EQ.LIT(5).OR.NDUM(3).EQ.LIT(5))
                  GO TO 749
      IF(NDUM(2).LE.0.OR.NDUM(4).LE.0)  GO TO 745
      IF(NDUM(5).EQ.LIT(5).AND.NDUM(7).NE.LIT(1))
                  GO TO 749
      IF(NDUM(6).LT.0)  GO TO 745
      IF(NDUM(8).LT.0.AND.NDUM(7).NE.LIT(5))  GO TO 745
      IF(NDUM(8).LT.0)  GO TO 730
      IF(NDUM(9).NE.LIT(1))  GO TO 730
C
C     ESTABLISH THE CATEGORY.    IF(KSW.EQ.0)
                  MAKE A NEW START.
C
      IF(KSW.NE.0)  GO TO 720
      IF(NDUM(2).NE.1)  GO TO 730
      IF(NDUM(4).EQ.2)  GO TO 711
      KSW = 87
      IF(NDUM(4).NE.1.OR.NDUM(6).NE.1)  GO TO 730
      IF(NDUM(8).EQ.0.AND.NDUM(7).EQ.LIT(1))  GO TO 780
      IF(NDUM(7).NE.LIT(1).AND.NDUM(7).NE.LIT(5))
                  GO TO 730
      IF(NDUM(8).LE.2.AND.NDUM(7).EQ.LIT(5))  GO TO 780
      GO TO 730
  711 IF(NDUM(6).EQ.0.AND.NDUM(8).EQ.0)  GO TO 750
      IF(NDUM(6).EQ.2.AND.NDUM(5).EQ.LIT(5))  GO TO 750
      GO TO 730
C
C
C
  720 IF(KSW.EQ.31)  GO TO 723
      IF(NDUM(2).NE.1)  GO TO 730
      IF(KSW.NE.52)  GO TO 722
      IF(NDUM(4).NE.2)  GO TO 721
      IF(NDUM(8).NE.0)  GO TO 745
      IF(NDUM(6).EQ.0)  GO TO 752
      IF(NDUM(6).EQ.2.AND.NDUM(5).EQ.LIT(5))  GO TO 752
```

```
      GO TO 745
  721 IF(NDUM(4).NE.1.AND.NDUM(6).NE.1)  GO TO 745
      IF(NDUM(8).EQ.0)  GO TO 760
      IF(NDUM(8).LE.2.AND.NDUM(7).EQ.LIT(5))  GO TO 760
      GO TO 745
  722 IF(KSW.NE.87)  GO TO 725
  723 IF(NDUM(4).NE.1.OR.NDUM(6).NE.1)  GO TO 730
      KSW = 87
  724 IF(NDUM(8).EQ.0)  GO TO 780
      IF(NDUM(8).LE.2.AND.NDUM(7).EQ.LIT(5))  GO TO 780
      GO TO 730
  725 IF(KSW.NE.61)  GO TO 745
      IF(NDUM(4).NE.1.AND.NDUM(6).NE.1)  GO TO 745
      GO TO 724
C
C     OUTPUT UNCATEGORISABLE MIXED SPECIES.
C
  730 KSW = 31
  731 IF(KSW.GE.50.AND.KSW.LT.80)  GO TO 741
      IF(BLOCK(NDUM,PURGE,NND,NKEY,NPURGE,LIT(1)))
                  GO TO 701
      IF(IPRT) WRITE(OUT,30731)  XA, (NDUM(N), N=1,NN)
C      ************************************************
                  ********
C     WRITE(OUTF,20401) XA, (NDUM(N), N=1,NN)
      NNX = NN + 1
      IF(NNX.GE.NND)  GO TO 435
      DO 434 N=NNX,NND,2
      NDUM(N) = LIT(1)
  434 NDUM(N+1) = 0
  435 WRITE(OUTF,20401)  XA, NDUM, LIT(1)
C      ************************************************
                  ********
      NTOT = NTOT + 1
      NSECC = NSECC -1
      IF(NMET.EQ.1)  MAXMIX = MAXMIX - 1
      GO TO 701
C
C     OMIT THE SPECIES.
C
  740 IF(IPRT) WRITE(OUT,30741)
      IF(CHEK(NDUM(N1),PCOMP,NP).EQ.-1)  GO TO 9997
  741 IF(IPRT) WRITE(OUT,30742)  NNN
      IF(KSW.NE.0)  GO TO 701
      IF(NDUM(4).NE.2)  GO TO 701
      IF(NDUM(6).EQ.0.OR.(NDUM(5).EQ.LIT(5).AND.NDUM(6)
                  .EQ.2)) KSW = 100
      GO TO 701
C
C     ERROR EXIT
C
  745 WRITE(OUT,30745)  NNN
      WRITE(OUT,30746)  KSW
      GO TO 9999
  746 WRITE(OUT,30747) NNN
```

```
      GO TO 9999
  747 WRITE(OUT,30748)
      GO TO 745
  749 WRITE(OUT,30749)
      GO TO 745
C
C
C
  750 DO 751 I=1,NMH
  751 CBUF(I) = -1001.0
      KSW = 52
C
C     IDENTIFY THE LIGAND.   IF(KSW.NE.71) FILL CBUF FOR
                  PREDICTION LATER.
C
  752 IF(NDUM(5).EQ.LIT(5))  GO TO 753
      N1 = 1
      N2 = NM
      GO TO 754
  753 N1 = NM + 1
      N2 = NMH
  754 DO 755 I=N1,N2
      IF(NDUM(3).EQ.MCOMP(I))  GO TO 756
  755 CONTINUE
      IF(KSW.EQ.71)  GO TO 775
      GO TO 741
  756 IF(CBUF(I).GT.-100.0)  GO TO 745
      IF(KSW.EQ.71)  GO TO 771
      NB = I
      CBUF(I) = XA / 2.0
      GO TO 701
C
C
C
  760 IF(KSW.NE.52)  GO TO 745
      KSW = 61
      GO TO 780
C
C     PREDICT TERNARY STABILITY CONSTANT FROM THE BINARY
                  VALUES IN CBUF
C    . WHICH HAVE BEEN DETERMINED UNDER NON-PHYSIOLOGICAL
                  CONDITIONS.
C
  761 IF(LX.LE.NM.AND.LY.LE.NM)  GO TO 768
      IF(LX.GT.NM.AND.LY.GT.NM)  GO TO 768
C
C    IF THERE ARE TWO WAYS OF PREDICTING, USE THE ONE
                  WHICH IS CLOSEST.
C
      IF(ND.EQ.0)  GO TO 767
      IXA = MIN0(LX,LY)
      IXB = LY
      IF(IXA.EQ.LY)  IXB = LX
      N1 = 0
      N2 = 0
```

```
      DO 762 I=1,ND
      IF(DUP(I,1).EQ.IXB)  N1 = DUP(I,2)
      IF(DUP(I,2).EQ.IXA)  N2 = DUP(I,1)
  762 CONTINUE
      IF(N1.EQ.0.OR.N2.EQ.0)  GO TO 767
      IF(CBUF(N1).LT.-100.0.OR.CBUF(N2).LT.-100.0)
                  GO TO 767
      IF(CBUF(IXA).GT.-100.0.AND.CBUF(IXB).GT.-100.0)
                  GO TO 764
  763 LX = N2
      LY = N1
      GO TO 767
  764 AXA = XA - (CONSTM(IXA) + CONSTM(IXB))
      XB = XA - (CONSTM(N1) + CONSTM(N2))
      AXA = ABS(AXA)
      XB = ABS(XB)
      IF(AXA.GT.XB)  GO TO 763
  767 CMIX(J) = CONSTM(LX) + CONSTM(LY)
      IF(CBUF(LX).GT.-100.0.AND.CBUF(LY).GT.-100.0)
                  GO TO 768
      WRITE(OUT,30761)
      GO TO 745
  768 XB = XA - (CBUF(LX) + CBUF(LY))
      IF(IPRT) WRITE(OUT,30763)  (NDUM(N), N=1,NN)
      IF(IPRT) WRITE(OUT,30764)  CBUF(LX), CBUF(LY), XB,
                  XA
      IF(STAB(J).LT.500.0)  GO TO 747
      STAB(J) = XB * ((CONSTM(LX) + CONSTM(LY)) /
                  (CBUF(LX) + CBUF(LY)))
      XB = CMIX(J) + STAB(J)
      IF(SUPRES)  GO TO 701
      WRITE(OUT,30764)  CONSTM(LX), CONSTM(LY), STAB(J),
                  XB
      GO TO 701
C
C     CATEGORISE SPECIES FOR STABILISATION FACTOR
                  SUBSTITUTION (KSW=71).
C
  770 IF(NDUM(3).EQ.LIT(1))  GO TO 700
      IF(KSW.EQ.100)  NT = NT + 1
      IF(KSW.EQ.0.OR.KSW.EQ.100)  GO TO 701
      IF(KSW.NE.52.AND.KSW.NE.71.AND.KSW.NE.72)  GO TO 745
      NT = NT + 1
      KSW = 71
      LX = NB
      XB = CONSTM(LX) / CBUF(LX)
      GO TO 752
C
C     PREPARE FOR STABILISATION FACTOR SUBSTITUTION (BY
                  ADJUSTMENT MECHANISM).
C
  771 LY = I
      CBUF(I) = XA
      KSW = 72
      GO TO 785
```

```
C
C       ADJUSTMENT MECHANISM.    XA = STAB. FACTOR;
                    XB = CONDITIONS RATIO.
C
  772 IF(STAB(J).LT.500.0)  GO TO 747
      STAB(J) = XA * XB
      N1 = 0
      IF(LX.GT.NM)  N1 = N1 + 1
      IF(LY.GT.NM)  N1 = N1 + 1
      IF(SUPRES)  GO TO 701
      WRITE(OUT,30771)  NCOMP(METAL),MCOMP(LX),MCOMP(LY),
                    N1,XA,STAB(J)
      GO TO 701
C
C       DECIDE WHY THE SPECIES IS TO BE OMITTED.
C
  775 DO 776 I=1,NIII
      IF(NDUM(3).EQ.NCOMP(I))  GO TO 741
  776 CONTINUE
      N1 = 3
      GO TO 740
C
C       DETERMINE THE INDEXES LX AND LY FOR THE COMPONENTS
                    IN NDUM(3) AND NDUM(5).
C
  780 IF(NDUM(3).EQ.NDUM(5))  GO TO 745
      LX = 0
      LY = 0
      IF(NDUM(8).EQ.0)  GO TO 782
      N1 = NM + 1
      DO 781 I=N1,NMH
      IF(NDUM(3).EQ.MCOMP(I))  LX = I
      IF(NDUM(5).EQ.MCOMP(I))  LY = I
  781 CONTINUE
      IF(NDUM(8).EQ.2)  GO TO 785
      IF(LX.EQ.0.AND.LY.EQ.0)  GO TO 731
      N2 = 0
      N3 = 0
      IF(LX.EQ.0.OR.LY.EQ.0)  GO TO 782
      N1 = MIN0(LX,LY)
      N2 = LX
      N3 = LY
      LX = 0
      LY = 0
  782 DO 783 I=1,NM
      IF(LX.EQ.0.AND.NDUM(3).EQ.MCOMP(I))  LX = I
      IF(LY.EQ.0.AND.NDUM(5).EQ.MCOMP(I))  LY = I
  783 CONTINUE
      IF(NDUM(8).EQ.0)  GO TO 785
      IF(LX.EQ.0.OR.LY.EQ.0)  GO TO 784
      IF(N1.EQ.N2)  LX = N2
      IF(N1.EQ.N3)  LY = N3
      GO TO 785
  784 IF(LX.EQ.0.AND.LY.EQ.0)  GO TO 731
      IF(LX.EQ.0)  LX = N2
```

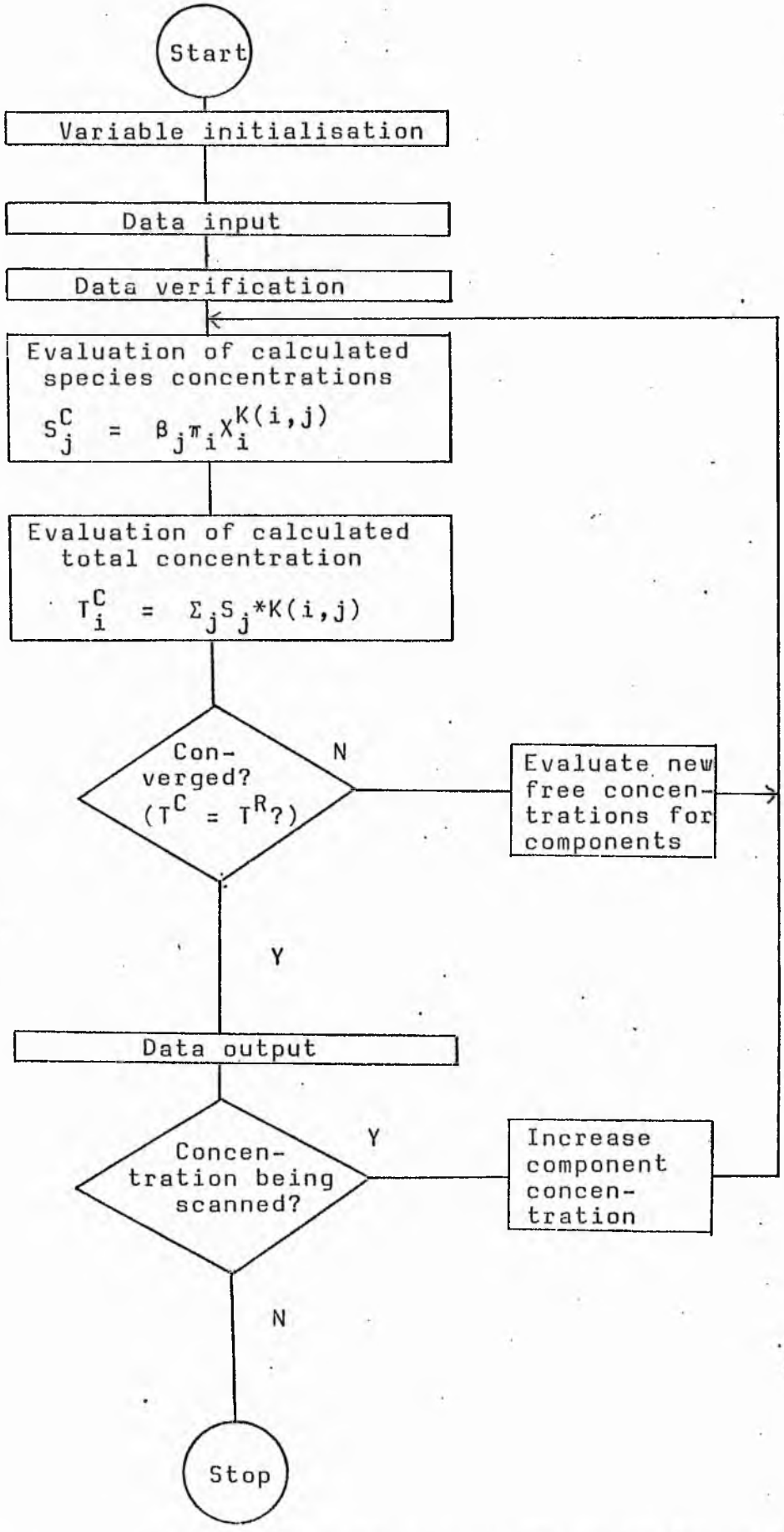
```
      IF(LY.EQ.0)  LY = N3
C
C     CALCULATE THE MIXED SPECIES INDEX AND CORRECT IT IF
                  NECCESSARY.
C
  785 IF(LX.EQ.0.OR.LY.EQ.0)  GO TO 731
      L = MAX0(LX,LY)
      M = L - 1
      I = LX
      IF(I.EQ.L)  I = LY
      L = M - 1
      J = ((M * L) / 2) + I
      IF(NZ.EQ.0)  GO TO 787
      DO 786 I=1,NZ
      IF(J.EQ.OMIT(I,1))  J = OMIT(I,2)
  786 CONTINUE
  787 IF(J.LE.0)  GO TO 745
      IF(KSW.EQ.72)  GO TO 772
      IF(KSW.EQ.61)  GO TO 761
      IF(KSW.NE.87)  GO TO 745
C
C     DIRECT STABILITY CONSTANT SUBSTITUTION.
C
      IF(STAB(J).LT.500.0)  GO TO 747
      STAB(J) = XA - CMIX(J)
      CMIX(J) = XA - STAB(J)
      IF(SUPRES)  GO TO 701
      WRITE(OUT,30781)  XA, (NDUM(N), N=1,NN)
      GO TO 701
C
C
C
C     SECTION EIGHT       OUTPUT THE CALCULATED MIXED
                  LIGAND FORMATION CONSTANTS.
C
C
  800 REWIND IN3
      NN = NMX + NZ
      DO 801 I=1,NN
      LGMIX(J) = 0.0000
      IF(STAB(I).GT.500.0)  STAB(I) = STABF
  801 CMIX(I) = CMIX(I) + STAB(I)
C
C     THE PRE-SCAN.
C
      NNN = NTOT
      FACNEW = -20.00
      IF(NMX.LE.MAXMIX)  GO TO 899
      IF(ERCONC)  GO TO 9994
      FACMAX = 0.0000
      FACMIN = -500.0
      J = 0
      DO 893 M=2,NMM
      N = M - 1
      DO 893 I=1,N
```

```
      J = J + 1
      AXA = AMIN1(TOTLM,TREALM(M),TREALM(I))
      LGMIX(J) = CMIX(J) + FREEM + XXM(M) + XXM(I) - AXA
      IF(SUPRES)  GO TO 893
      IF(M.LE.NM)  GO TO 892
      IF(OMIT(1,1).EQ.0)  GO TO 892
      DO 890 K=1,NZ
      IF(OMIT(K,1).EQ.J)  GO TO 891
  890 CONTINUE
      GO TO 892
  891 IF(OMIT(K,2).EQ.0)  GO TO 893
      WRITE(OUT,30892)  J, I, M, CMIX(J), OMIT(K,2)
      GO TO 893
  892 WRITE(OUT,30893) J,I,M,CMIX(J),STAB(J),XXM(I),
                   XXM(M),AXA,LGMIX(J)
  893 CONTINUE
  894 IXA = 0
      DO 895 I=1,NN
      IF(LGMIX(I).GT.FACNEW)  IXA = IXA + 1
  895 CONTINUE
      IF(IXA - MAXMIX)  896,899,897
  896 FACMAX = FACNEW
      GO TO 898
  897 FACMIN = FACNEW
  898 FACNEW = (FACMAX + FACMIN) / 2.0
      IF(FACMAX - FACNEW.GT.0.00001)  GO TO 894
      WRITE(OUT,30899)  MAXMIX
      FACNEW = FACMAX
  899 NDUM(1) = NCOMP(METAL)
      NDUM(2) = 1
      NDUM(3) = MCOMP(1)
      NDUM(4) = 1
      NDUM(5) = MCOMP(2)
      NDUM(6) = 1
      J = 0
      DO 804 M=2,NMM
      N = M - 1
      DO 804 I=1,N
      J = J + 1
      NDUM(3) = MCOMP(I)
      NDUM(5) = MCOMP(M)
      NN = 6
      IF(LGMIX(J).LE.FACNEW)  GO TO 804
      IF(M.LE.NM)  GO TO 803
      NN = 8
      NDUM(7) = LIT(5)
      NDUM(8) = 1
      IF(I.GT.NM) NDUM(8) = 2
      IF(BLOCK(NDUM,PURGE,NND,NKEY,NPURGE,LIT(1)))
                   GO TO 804
      IF(OMIT(1,1).EQ.0)  GO TO 803
      DO 802 K=1,NZ
      IF(OMIT(K,1).EQ.J)  GO TO 804
  802 CONTINUE
  803 NTOT = NTOT + 1
```

```
C      ***************************************************
                    ********
C      WRITE(OUTF,20401)  CMIX(J), (NDUM(N1), N1=1,NN)
       NNX = NN + 1
       IF(NNX.GE.NND)  GO TO 806
       DO 805 N1=NNX,NND,2
       NDUM(N1) = LIT(1)
  805  NDUM(N1+1) = 0
  806  WRITE(OUTF,20401)  CMIX(J), NDUM, LIT(1)
C      ***************************************************
                    ********
  804  CONTINUE
       NNN = NTOT - NNN
       NSECC = NSECC - NNN
       NREJEK = NREJEK + NMX - NNN
       WRITE(OUT,30801)  NMX, NCOMP(METAL), NNN, FACNEW
       WRITE(OUT,30802)  NT
       GO TO 500
C
C
C
C      SECTION NINE       FINISH!
C
C
  900  NNN = NTOT + NREJEK
       NT = NTOT
       NTOT = NTOT + NIII
       WRITE(OUT,30901) NTOT
       IF(NREJEK.GT.0)  WRITE(OUT,30903)  NT, NNN
       GO TO 901
 9994  WRITE(OUT,30994)
       GO TO 9999
 9995  WRITE(OUT,30995)
       GO TO 9999
 9996  WRITE(OUT,30996)  NDUM(ND)
       GO TO 9998
 9997  WRITE(OUT,30997)  NDUM(N1)
 9998  WRITE(OUT,30998)  NNN
 9999  WRITE(OUT,30999)
       ENDFILE OUTF
       NT = 0
       REWIND OUTF
       WRITE(OUTF,20999)
       CALL EXIT(3)
  901  WRITE(OUT,30902)
       ENDFILE OUTF
       STOP
       END
-------------------------------------------------------------
       INTEGER FUNCTION CHEK(NCHEK,PCOMP,NP)
       INTEGER  PCOMP(NP)
       CHEK = 1
       DO 100 I=1,NP
       IF(NCHEK.EQ.PCOMP(I))  RETURN
  100  CONTINUE
       CHEK = -1
```

```
      RETURN
      END
      LOGICAL FUNCTION BLOCK(NDUM,PURGE,NND,NKEY,NPURGE,
     1           LIT)
      INTEGER  NDUM(NND), PURGE(NKEY,NND)
      BLOCK = .FALSE.
      IF(NPURGE.LT.1)  RETURN
      DO 104 IPURGE=1,NPURGE
      DO 102 IKEY=1,NKEY
      IF(PURGE(IKEY,IPURGE).EQ.LIT)  GO TO 103
      DO 101 IND=1,NND,2
      IF(PURGE(IKEY,IPURGE).EQ.NDUM(IND))  GO TO 102
  101 CONTINUE
      GO TO 104
  102 CONTINUE
  103 BLOCK = .TRUE.
      RETURN
  104 CONTINUE
      RETURN
      END
```

FLOW DIAGRAM FOR PROGRAM ECCLES



```
C
C       PROGRAM ECCLES.
C       ---------------
C
C
C       DEVELOPED AT THE UNIVERSITY OF CAPE TOWN DURING
                    1974 AND 1975.
C       RE-WRITTEN AT THE UNIVERSITY OF ST. ANDREWS DURING
                    1976 AND 1977.
C       MODIFIED AT THE UNIVERSITY OF WALES INSTITUTE OF
                    SCIENCE AND
C       TECHNOLOGY DURING 1978 AND 1979.
C
C       THIS PROGRAM EVALUATES THE CONSTITUENT
                    CONCENTRATIONS IN LARGE
C       EQUILIBRIUM SYSTEMS.   IN ADDITION, IT IS DESIGNED
                    TO REFLECT THE MORE
C       PRONOUNCED EFFECTS OF SYSTEMATIC CONCENTRATION
                    CHANGES IN THE
C       EQUILIBRIUM MIXTURE.
C
C       THE PROGRAM IS WRITTEN IN FORTRAN IV.
C
C
C
C
C       SECTION ONE.        STORAGE ALLOCATION AND FORMAT
                    STATEMENTS
C
C
C
        DOUBLE  PRECISION  CONST(5000),  SPECIE(5000),
                    TIE(2)
        DIMENSION KEY(18000), NUMT(5000), NUMF(5000),
                    NST(5000), NSF(5000)
        DIMENSION UNIQ(5,5000)
        DIMENSION   NCOMP(110), X(100), XX(15), FDASH(100),
                    TREAL(100),
       1 TCALC(115), BETALG(5000), JSP(5000), IMON(15),
                    LIT(15), SPC(5000)
        INTEGER  CHECK(115), TITLE(18), NAME(9), DATE(9),
                    OUT, CARD,
       1   SIMUL(10)
        REAL LGKWD, PMI(15), XXLOG(15), POWER(100)
        LOGICAL MONIT,MONALL,SCAN,MULTIP,SUPRES,TRIAL,
                    UCHECK,SWTCH,PUNCH
C
C
C       INTEGER*2      KEY, NUMT, NUMF, NST, NSF, UNIQ
C
C
C
        EQUIVALENCE (CONST(1), UNIQ(1,1), JSP(1))
        EQUIVALENCE (CONST(5000), TIE(1)), (SPECIE(1),
                    TIE(2))
```

```
      EQUIVALENCE (TITLE(1), XXLOG(1))
      DATA   LIT/'    ','ABOR','ALL ','XXXX','SUPR',
                 'TEST','OMIT','TOTA'
     1 ,'END ','SPEC','FREE','H +1','OH-1','SWIT','UNCH'/
C
C     THE PARAMETER VARIABLES.
C
      NK = 18000
      NS = 5000
      NX = 100
      NXX = 15
      NXXX = NX + NXX
      NLINES=63
C
C     THE FORMAT STATEMENTS.
C
10300 FORMAT(18A4)
10301 FORMAT(9A4)
10302 FORMAT(L5,1X,14(A4,1X),A4)
10303 FORMAT(L5,1X,A4,2G10.4,10(1X,A4))
10311 FORMAT(A4,A1,2G10.4)
10321 FORMAT(G9.5,1X,5(A4,1X,I2,2X),8A1)
20000 FORMAT('1',/'0')
20301 FORMAT(//'0',18A4,///)
20302 FORMAT('1',5(1H*),2(6X,4(1H*)),5X,1H*,4X,2(5X,
                5(1H*)),/' ',
     1 6(1H*,9X),/' ',4(1H*),6X,3(1H*,9X),4(1H*),6X,
                5(1H*),/' '
     2 5(1H*,9X),4X,1H*,/' ',5(1H*),2(6X,4(1H*)),3(5X,
                5(1H*)),/' ',
     3 55(1H-),//////'0',T25,40(2H<>),2(/'
                ',T25,2H<>,T103,2H<>,
     4 /' ',T25,4H<>   18A4,4H  <>,/' ',T25,2H<>,T103,
                2H<>),/' ',T25,
     5 40(2H<>),////)
20303 FORMAT('0','OUTPUT SUPRESSED.')
20304 FORMAT('0','COMPLETE OUTPUT REQUIRED.')
20305 FORMAT(' ','MONITORED COMPONENTS:  ',15(A4,1X))
20306 FORMAT(' ','NO COMPONENTS MONITORED.')
20307 FORMAT(' ','SCAN COMPONENT:  ',A4,/' ','SCAN
                INCREMENT =',1PE12.4,
     1 5X,'MAX. VALUE =',1PE12.4)
20308 FORMAT(' ','MULTIPLICATIVE INCREMENT')
20309 FORMAT(' ','ADDITIVE INCREMENT')
20310 FORMAT(' ','NO SCAN REQUIRED.')
20311 FORMAT(' ','NO DATA CHECK REQUIRED.')
20312 FORMAT(' ','ECCLES DATA CHECKING PROCEDURES
                EMPLOYED.')
20313 FORMAT('0','THE MAXIMUM NUMBER OF VARIABLE
                COMPONENTS IS',I3)
20314 FORMAT('0','**********  WARNING  **********',/'
                ','IF THE SOLUTION
     1 IS AQUEOUS A FREE HYDROGEN ION CONCENTRATION IS
                REQUIRED.',//)
```

```
20315 FORMAT('0','THE MAXIMUM NUMBER OF FIXED COMPONENTS
                  IS',I3)
20316 FORMAT(' ','SIMULTANEOUS SCAN OF',2X,16(A4,1X))
20321 FORMAT('0','INCORRECT NEGATIVE SUBSCRIPT. SPECIES
                  NUMBER',I5,
     1 ' IS IN ERROR.')
20322 FORMAT('0','A FREE HYDROXYL ION CONCENTRATION IS
                  REQUIRED.')
20331 FORMAT('0','THE MAXIMUM NUMBER OF COMPLEX SPECIES
                  IS',I5)
20350 FORMAT('0','TOO MANY COMPONENTS.')
20351 FORMAT('0','THE PROGRAM LIMITS HAVE BEEN EXCEEDED.')
20352 FORMAT('0','EXECUTION TERMINATED.
                  ERROR ON CARD',I5)
20353 FORMAT(' ','SPECIES NUMBER',I5)
20354 FORMAT('0','TEST AND SCAN OPTIONS INCOMPATIBLE.')
20355 FORMAT('0','MISPLACED FIELD?')
20356 FORMAT('0','COMPONENT DUPLICATION.
                  COMPONENT',A4,' HAS ALREADY B
     1EEN ENTERED.')
20357 FORMAT('0','COMPONENT ERROR DETECTED.')
20358 FORMAT('0','SPECIES MUST HAVE AT LEAST TWO
                  COMPONENTS.')
20360 FORMAT('0','A POSITIVE CONCENTRATION FOR COMPONENT
                  ',A4,
     1 ' IS REQUIRED.')
20370 FORMAT('1','DUMP OF ARRAYS TO ESTABLISH EXTENT OF')
20371 FORMAT(//'0','COMPONENT SYMBOLS AND
                  CONCENTRATIONS',//)
20372 FORMAT(' ',I4,5X,A4,2X,1PE15.4)
20373 FORMAT(//'0','SPECIES ARRAY :',8X,I6,1PE15.4,5X,
                  5(A4,I3,3X),////)
20375 FORMAT('0','PREVIOUS BETA VALUE:  ',I4,1PE15.4)
20401 FORMAT(' ','THERE ARE',I3,' SPECIES WITH DEFINED
                  CONCENTRATIONS.')
20421 FORMAT('0','SPECIES DUPLICATION.
                  SPECIES NUMBERS',2I6,
     1   '  ON CARD NUMBERS',2I6)
20441 FORMAT('0','UNIDENTIFIABLE SCAN COMPONENT.')
20451 FORMAT('0','UNIDENTIFIABLE MONITOR COMPONENT.')
20452 FORMAT('0','A MONITORED COMPONENT MUST APPEAR IN AT
                  LEAST THREE SP
     1ECIES',/' ',A4,' HAS BEEN REMOVED FROM THE
                  LIST.',//)
20470 FORMAT('0','EXECUTION TERMINATED.',/' ','COMPONENT
                  NUMBER',I3,
     1' IS NEVER REFERENCED.',//)
20471 FORMAT('0','THE MAXIMUM NUMBER OF COMPONENT
                  ENTITIES IN THE LIST O
     1F SPECIES IS',I6,/'0','EXECUTION TERMINATED.')
20472 FORMAT('0','EXECUTION TERMINATED.
                  ERROR COUNT =',I4)
20474 FORMAT('0','THE SCAN INCREMENT FACTOR IS TOO
                  SMALL.')
```

```
20475 FORMAT('0','THE MAXIMUN SCAN CONCENTRATION IS LOWER
                 THAN THE START
     1ING VALUE.')
20476 FORMAT(' ','THE CONCENTRATION OF THE SCANNED
                 COMPONENT MUST INCREA
     1SE.')
20480 FORMAT('0','DATA INPUT SUCCESSFULLY COMPLETED.',/'
                 ',
     1'TOTAL NUMBER OF COMPONENTS =',I4,2X,'(LIMIT
                 =',I4,' )',/' ',
     2'NUMBER OF COMPONENTS WITH FIXED FREE
                 CONCENTRATIONS =',I3,
     3 2X,'(LIMIT =',I3,' )',/' ',
     4'TOTAL NUMBER OF SPECIES =',I6,2X,'(LIMIT =',I6,'
                 )',/' ',
     5'TOTAL NUMBER OF COMPONENTS ENTERED IN KEY ARRAY
                 =',I6,
     6 2X,'(LIMIT =',I6,' )')
20481 FORMAT('0','EXPERIMENTAL PH =',F12.3)
20482 FORMAT('+',T45,'CALCULATED LOG KW =',F12.4)
20485 FORMAT(//'0','ITERATION RECORD',/' ',16(1H-),//
     1' TOTAL AND FREE CONCENTRATIONS OF THE FIRST FEW
                 COMPONENTS',//)
20701 FORMAT(' ','CYCLE',I4,6X,3(1PE12.4,2X,'(',E10.4,')',
                 5X))
20801 FORMAT('0','**********   ITERATION LIMIT EXCEEDED.
                  **********')
21000 FORMAT('1',T50,'ECCLES: SCAN CYCLE NUMBER',I3,/'
                 ',T50,30(1H*)///)
21001 FORMAT(1H1)
21002 FORMAT('0','CONCENTRATIONS OF THE FREE
                 COMPONENTS.',/' ',37(1H-)/)
21003 FORMAT('0 COMP.',T14,'FREE',T34,'REAL',T54,'CALC.',
                 T72,'COMP.',
     1 T84,'ITERATION',12X,'LOG',/
     2 3X,'NO.',T14,'CONC.',T34,'TOTAL',T54,'TOTAL',T72,
                 'NAME',T83,
     4 'INFORMATION',10X,'P.M.I.',//)
21004 FORMAT(' ',I4,2X,3(1PE15.6,5X),T72,A4,I8,3X,0PF7.2)
21005 FORMAT(' ',I4,2X,1PE15.6,6X,'FIXED FREE
                 CONC.',3X,1PE15.6,5X,T72,
     1 A4,I8,18X,0PF7.2)
21011 FORMAT(' ','CONCENTRATIONS OF THE SPECIES.',/'
                 ',29(1H-),///)
21012 FORMAT(' ',' SPECIES',T14,'SPECIES',T32,'LOG
                 STAB.',T50,
     1'COMPOSITION.',/4X,'NO.',T15,'CONC.',T34,'CONST.',
                 //)
21021 FORMAT(' ',I5,1PE15.4,5X,0PF12.3,T50,10(A4,'(',I1,
                 ') '))
21022 FORMAT(/'0','THE SOLUTION WAS REACHED AFTER',I5,'
                 ITERATIONS.')
21023 FORMAT('0','EXECUTION TERMINATED WITHOUT
                 CONVERGENCE.')
21024 FORMAT('0','THE PRECISION LIMIT WAS ',1PG10.3)
```

```
21101 FORMAT('1','MONITOR COMPONENT ',A4,T50,'FREE CONC.
                  =',
     1  1PE12.4,0PF7.1,'%',/' ',22(1H-),//)
21131 FORMAT(' ',I6,1PE14.4,0PF6.1,'%',2X,F8.2,T50,10(A4,
                  '(',I1,') '))
31100 FORMAT(A4,5X,2G15.5)
31101 FORMAT(G15.5,3X,10(A4,I1,1X))
99999 FORMAT('1',34H'WELL DONE ECCLES', SAID
                  MORIARTY.,/////)
C
C
C     SECTION TWO.     INITIALSATION.
C
C
  200 IN = 5
      ISWTCH = 9
      OUT=6
      CARD = 7
      NN=4
      NXP1 = NX + 1
      NXXP1 = NXX + 1
      NSP1 = NS + 1
      MULTIP = .FALSE.
      SUPRES=.FALSE.
      TRIAL=.FALSE.
      MONALL=.FALSE.
      UCHECK=.FALSE.
      SWTCH = .FALSE.
      PUNCH = .FALSE.
      LGKWD=0.00000
      DO 201 I=1,NK
  201 KEY(I) = 0
      DO 202 I=1,NXXX
  202 CHECK(I) = 0
      DO 203 J=1,NS
      DO 203 I=1,5
  203 UNIQ(I,J) = 0
      DO 204 I=1,NX
  204 POWER(I) = 0.00
      NKT=1
      NKF=NK
      NJ=0
      NCYCLE=1
      NSTOP = 800
      NSUPF = 0
      TLIM = 1.5E-6
C
C
C     SECTION THREE.     INPUT.
C
C
      READ(IN,10300) TITLE
      IF(TITLE(1).EQ.LIT(2))  WRITE(OUT,20301)  TITLE
      IF(TITLE(1).EQ.LIT(2))  GO TO 9999
      READ(IN,10301)  NAME, DATE
```

```
      READ(IN,10302,ERR=352)  MONIT, IMON
      IF(IMON(1).EQ.LIT(3))  MONALL = .TRUE.
      IF(IMON(1).EQ.LIT(15))  PUNCH = .TRUE.
      NN=5
      READ(IN,10303,ERR=352) SCAN, ISCN, SCNINC, SCNMAX,
     1            SIMUL
      IF(SCAN.AND.SCNINC.GT.1.0)  MULTIP = .TRUE.
      NN=6
      READ(IN,10301) IXA
      IF(IXA.EQ.LIT(5))  SUPRES = .TRUE.
      IF(.NOT.SUPRES) GO TO 301
      READ(IN,10301) IXA
      NN=NN+1
  301 IF(IXA.EQ.LIT(6))  TRIAL = .TRUE.
      IF(.NOT.TRIAL) GO TO 302
      READ(IN,10301) IXA
      NN=NN+1
      IF(ISCN.EQ.LIT(6))  NSTOP = IFIX(SCNINC)
      IF(NSTOP.LT.0.OR.NSTOP.GT.1000) GO TO 354
  302 IF(IXA.EQ.LIT(7))  UCHECK = .TRUE.
      IF(.NOT.UCHECK) GO TO 303
      READ(IN,10301) IXA
      NN=NN+1
  303 IF(IXA.EQ.LIT(14))  SWTCH = .TRUE.
      IF(SWTCH)  READ(IN,10301)  IXA
      IF(SWTCH)  NN = NN + 1
      IF(IXA.NE.LIT(8))  GO TO 352
C
C     OUTPUT IDENTIFICATION.
C
      IF(DATE(1).EQ.LIT(1).AND.DATE(2).EQ.LIT(1))
     1            GO TO 306
  304 IF(DATE(9).NE.LIT(1))  GO TO 306
      M=10
      DO 305 L=1,8
      M=M-1
      N=M-1
  305 DATE(M)=DATE(N)
      DATE(1) = LIT(1)
      GO TO 304
  306 IF(TITLE(17).NE.LIT(1).OR.TITLE(18).NE.LIT(1))
     1            GO TO 310
      M=17
      L=1
  307 L=L+1
      IF(L.EQ.18) GO TO 310
      M=M-1
      IF(TITLE(M).EQ.LIT(1))  GO TO 307
      IXA=18-(L / 2)
  308 IF(TITLE(IXA).NE.LIT(1))  GO TO 310
      M=19
      DO 309 L=1,17
      M=M-1
      N=M-1
  309 TITLE(M)=TITLE(N)
```

```
      TITLE(1) = LIT(1)
      GO TO 308
  310 WRITE(OUT,20302) TITLE, NAME, DATE
      IF(SUPRES)  WRITE(OUT,20303)
      IF(.NOT.SUPRES)  WRITE(OUT,20304)
      M = 1
      IF(PUNCH)  M = 2
      IF(MONIT)  WRITE(OUT,20305)  (IMON(I), I=M,15)
      IF(.NOT.MONIT)  WRITE(OUT,20306)
      IF(SCAN)  WRITE(OUT,20307) ISCN, SCNINC, SCNMAX
      IF(MULTIP)  WRITE(OUT,20308)
      IF(.NOT.MULTIP.AND.SCAN)  WRITE(OUT,20309)
      IF(.NOT.SCAN) WRITE(OUT,20310)
      IF(UCHECK)  WRITE(OUT,20311)
      IF(.NOT.UCHECK)  WRITE(OUT,20312)
C
C     INPUT COMPONENT DATA.
C
      IF(SCAN.AND.SIMUL(1).NE.LIT(1))  WRITE(OUT,20316)
     1            SIMUL
      DO 312 NI=1,NX
      NN = NN + 1
      READ(IN,10311,ERR=352)  NCOMP(NI), IXA, TREAL(NI),
     1            X(NI)
      IF(NCOMP(NI).EQ.LIT(9))  GO TO 313
      IF(X(NI).LE.0.000)  X(NI) = TREAL(NI)
      IF(UCHECK)  GO TO 312
      IF(TREAL(NI).LE.0.00)  GO TO 359
      IF(IXA.NE.LIT(1))  GO TO 355
      IF(NI.EQ.1)  GO TO 312
      M = NI - 1
      DO 311 I=1,M
      IF(NCOMP(NI).EQ.NCOMP(I))  GO TO 356
  311 CONTINUE
  312 CONTINUE
      READ(IN,10311,ERR=352)  IXA
      NI = NI + 1
      IF(IXA.EQ.LIT(9))  GO TO 313
      N = NX
      WRITE(OUT,20313)  N
      GO TO 351
  313 NI = NI - 1
      NII = 0
      NH = 0
      NOH = 0
      NN = NN + 1
      READ(IN,10301)  IXA
      IF(IXA.EQ.LIT(10))  WRITE(OUT,20314)
  314 IF(IXA.EQ.LIT(10))  GO TO 318
      IF(IXA.NE.LIT(11))  GO TO 352
      N = NI
      DO 316 NII=1,NXX
      N = N + 1
      NN= NN + 1
```

```
      READ(IN,10311,ERR=352)  NCOMP(N), IXA, XX(NII),
                    PMI(NII)
      IF(NCOMP(N).EQ.LIT(9))  GO TO 317
      IF(UCHECK)  GO TO 316
      IF(XX(NII).LE.0.00)  GO TO 360
      IF(IXA.NE.LIT(1))  GO TO 355
      M=N-1
      DO 315 I=1,M
      IF(NCOMP(N).EQ.NCOMP(I))  GO TO 356
  315 CONTINUE
  316 CONTINUE
      READ(IN,10311,ERR=352)  IXA
      NII = NII + 1
      IF(IXA.EQ.LIT(9))  GO TO 317
      N = NXX
      WRITE(OUT,20315)  N
      GO TO 351
  317 NII = NII - 1
      READ(IN,10301)  IXA
      GO TO 314
  318 IF(NII.EQ.0)  GO TO 320
      I = NI
      DO 319 II=1,NII
      I = I + 1
      IF(NCOMP(I).EQ.LIT(12))  NH = II
      IF(NCOMP(I).EQ.LIT(13))  NOH = II
  319 CONTINUE
      IF(NH.EQ.0)  WRITE(OUT,20314)
      IF(NH.EQ.0)  GO TO 320
      PH = XX(NH)
      PH = -1.000 * ALOG10(PH)
      N = NH
      NH = NH + NI
      IF(NOH.EQ.0)  GO TO 320
      LGKWD = XX(N) * XX(NOH)
      LGKWD = ALOG10(LGKWD)
      NOH = NOH + NI
C
C     INPUT SPECIES CONSTANTS AND COMPOSITION.
C
  320 NIII = NI + NII
      IF(SWTCH)  IN = ISWTCH
      DO 339 NJ=1,NS
      NN = NN+1
      READ(IN,10321,ERR=352,END=400)  BETALG(NJ), TITLE
      NUMT(NJ) = 0
      NUMF(NJ) = 0
      IF(UCHECK)  GO TO 322
      DO 321 L=11,18
      IF(TITLE(L).NE.LIT(1))  GO TO 355
  321 CONTINUE
  322 IXA = 1
C
      DO 334 N=1,10,2
      IF(TITLE(N).EQ.LIT(1))  GO TO 335
```

```
  323 DO 324 M=IXA,NIII
      IF(TITLE(N).EQ.NCOMP(M))  GO TO 325
  324 CONTINUE
      IF(IXA.EQ.1)  GO TO 357
      IXA = 1
      GO TO 323
  325 CHECK(M) = CHECK(M) +1
      IXA = M
      L = N + 1
      KIJ = TITLE(L)
C
      IF(KIJ.GE.1)  GO TO 330
C
      IF(M.GT.NI.AND.TITLE(N).EQ.LIT(12))  GO TO 327
  326 WRITE(OUT,20321)  NJ
      IF(NH.EQ.0.AND.NOH.EQ.0)  NOH = NIII
      IF(NOH.EQ.0)  NOH = NH
      NOH = IABS(NOH)
      NOH = -NOH
      GO TO 328
  327 IF(KIJ.GT.-1.OR.KIJ.LT.-5)  GO TO 326
      IF(NOH.NE.0)  GO TO 328
      WRITE(OUT,20322)
      GO TO 326
  328 CHECK(M) = CHECK(M) - 1
      M = IABS(NOH)
      CHECK(M) = CHECK(M) + 1
      KIJ = IABS(KIJ)
      KIJ = MOD(KIJ,5)
      DO 329 L=1,KIJ
  329 BETALG(NJ) = BETALG(NJ) - LGKWD
C
  330 IML= N/2 + 1
      IF (.NOT. UCHECK) UNIQ(IML,NJ)= (KIJ * 1000) + M
      IF(M.GT.NI)  GO TO 332
      IF(FLOAT(KIJ).GT.POWER(M))  POWER(M) = FLOAT(KIJ)
      NUMT(NJ) = NUMT(NJ) + KIJ
      DO 331 L=1,KIJ
      KEY(NKT) = M
  331 NKT = NKT + 1
      GO TO 334
  332 NUMF(NJ) = NUMF(NJ) + KIJ
      DO 333 L=1,KIJ
      KEY(NKF) = M
  333 NKF = NKF - 1
  334 CONTINUE
C
      IF(.NOT.UCHECK)  GO TO 337
  335 IF(UCHECK)  GO TO 339
      DO 336 L=N,10,2
      IF(TITLE(L).NE.LIT(1))  GO TO 357
  336 CONTINUE
  337 N = N - 2
      IF(NUMT(NJ).EQ.0)  NSUPF = NSUPF + 1
      IF(N.LT.3)  GO TO 358
```

```
      DO 338 IXA=3,N,2
      KIJ = IXA - 2
      DO 338 L=1,KIJ,2
      IF(TITLE(L).EQ.TITLE(IXA))  GO TO 357
  338 CONTINUE
  339 CONTINUE
      READ(IN,10321,ERR=352,END=400)  XOLD
      WRITE(OUT,20331)  NJ
      GO TO 351
C
C     OUTPUT ERROR MESSAGES
C
  350 WRITE(OUT,20350)
  351 WRITE(OUT,20351)
  352 WRITE(OUT,20352)  NN
      IF(NJ.GT.0)  WRITE(OUT,20353)  NJ
      GO TO 370
  354 WRITE(OUT,20354)
      GO TO 352
  355 WRITE(OUT,20355)
      IF(NJ.GT.0)  GO TO 350
      GO TO 352
  356 WRITE(OUT,20356) NCOMP(N)
      GO TO 352
  357 WRITE(OUT,20357)
      GO TO 352
  358 WRITE(OUT,20358)
      GO TO 352
  359 N = NI
  360 WRITE(OUT,20360)  NCOMP(N)
      GO TO 352
C
C     DUMP ARRAY PARAMETERS AND CONTENTS.
C
  370 WRITE(OUT,20370)
      N = NKT + NK - NKF +1
      WRITE(OUT,20480)  NIII, NXXX, NII, NXX, NJ, NS, N,
                        NK
      WRITE(OUT,20371)
      DO 371 I=1,NI
  371 WRITE(OUT,20372)  I, NCOMP(I), TREAL(I)
      WRITE(OUT,20372)
      I = NI
      DO 372 N=1,NII
      I = I + 1
  372 WRITE(OUT,20372)  I, NCOMP(I), XX(N)
      IF(NJ.GT.0)  WRITE(OUT,20373)  NJ, BETALG(NJ),
                   (TITLE(N), N=1,10)
      NJ = NJ - 1
      IF(NJ.GT.0)  WRITE(OUT,20375)  NJ, BETALG(NJ)
      GO TO 9999
C
C
C     SECTION FOUR.    INPUT CHECKING PROCEDURES AND
                       OTHER PRELIMINARIES.
```

```
C
C
  400 NJ = NJ - 1
      NKT = NKT - 1
      NKF = NKF + 1
      KOUNT = NN
      IF(NSUPF.GT.0)  WRITE(OUT,20401)  NSUPF
      DO 401 I=1,NIII
      IF(CHECK(I).LE.0)  GO TO 470
  401 CONTINUE
      IXA = NKF - NKT
      IF(IXA.LE.0)  GO TO 471
      DO 402 I=1,NI
  402 POWER(I) = 1.000 / POWER(I)
C
C     TEST FOR SPECIES' UNIQUENESS.
C
      IF(UCHECK)  GO TO 440
      DO 413 J=1,NJ
      DO 412 I=1,4
      K = I + 1
      LAYBY = UNIQ(K,J)
      IF(LAYBY.EQ.0)  GO TO 413
      DO 411 L=1,I
      N = K - 1
      IF(LAYBY.LE.UNIQ(N,J))  GO TO 412
      UNIQ(K,J) = UNIQ(N,J)
  411 K = N
  412 UNIQ(K,J) = LAYBY
  413 CONTINUE
C
C
C
      K = 0
      DO 422 J=2,NJ
      N = J - 1
      LAYBY = UNIQ(1,J)
      DO 422 I=1,N
      IF(LAYBY.NE.UNIQ(1,I))  GO TO 422
      DO 421 L=2,5
      IF(UNIQ(L,J).NE.UNIQ(L,I))  GO TO 422
  421 CONTINUE
      L = KOUNT - NJ + I
      M = KOUNT - NJ + J
      K = K + 1
      WRITE(OUT,20421)  I, J, L, M
  422 CONTINUE
      IF(K.NE.0)  GO TO 472
C
C     NUMBER THE SCAN COMPONENT.
C
  440 IF(.NOT.SCAN)  GO TO 450
      DO 441 I=1,NIII
      IF(NCOMP(I).EQ.ISCN)  GO TO 443
  441 CONTINUE
```

```
      NN = 5
  442 WRITE(OUT,20441)
      GO TO 473
  443 IF(I.GT.NI)  II = I - NI
      IF(.NOT.MULTIP.AND.SCNINC.LE.0.000)  GO TO 474
      IF(MULTIP.AND.SCNINC.LE.1.000)  GO TO 474
      IF(I.LE.NI.AND.TREAL(I).GT.SCNMAX)  GO TO 475
      IF(I.GT.NI.AND.XX(II).GT.SCNMAX)  GO TO 475
      ISCN = I
C
C     NUMBER THE COMPONENTS SCANNED SIMULTANEOUSLY.
C
      DO 446 K=1,10
      IF(SIMUL(K).EQ.LIT(1))  GO TO 448
      DO 445 I=1,NIII
      IF(SIMUL(K).EQ.NCOMP(I))  GO TO 446
  445 CONTINUE
      GO TO 442
  446 SIMUL(K) = I
      K = 11
  448 NSIMUL = K - 1
C
C     NUMBER THE MONITORED COMPONENTS.
C
  450 IF(.NOT.MONIT.OR.MONALL)  GO TO 460
      L = 1
      IF(PUNCH)  L = 2
  451 N = 1
  452 DO 453 I=N,NIII
      IF(IMON(L).EQ.NCOMP(I))  GO TO 454
  453 CONTINUE
      IF(N.NE.1)  GO TO 451
      NN = 4
      WRITE(OUT,20451)
      GO TO 473
  454 N = I + 1
      IF(N.GT.NIII)  N = 1
      IF(CHECK(I).GE.3) GO TO 456
      WRITE(OUT,20452)  NCOMP(I)
      M = L + 1
      IF(M.GT.15)  GO TO 457
      IF(IMON(K).EQ.LIT(1))  GO TO 457
      DO 455 M = L,9
      IXA = M + 1
  455 IMON(M) = IMON(IXA)
      IMON(15) = LIT(1)
      GO TO 452
  456 IMON(L) = I
      L = L + 1
      IF(L.GT.15)  GO TO 457
      IF(IMON(L).NE.LIT(1))  GO TO 452
  457 NMON = L - 1
C
C
C
```

```
  460 IF(NOH.GE.0)  GO TO 480
      GO TO 9999
C
C     OUTPUT ERROR MESSAGES.
C
  470 WRITE(OUT,20470)  I
      GO TO 9999
  471 N = NK
      WRITE(OUT,20471)  N
      WRITE(OUT,20351)
      GO TO 9999
  472 WRITE(OUT,20472)  K
      GO TO 9999
  473 NJ = 0
      GO TO 352
  474 WRITE(OUT,20474)
      GO TO 476
  475 WRITE(OUT,20475)
  476 WRITE(OUT,20476)
      NN = 5
      GO TO 473
C
C     ACKNOWLEDGE THAT ALL'S WELL.
C
  480 N = NKT + NK - NKF + 1
      WRITE(OUT,20480)  NIII, NXXX, NII, NXX, NJ, NS, N,
     1                  NK
      IF(NH.GT.NI)  WRITE(OUT,20481)  PH
      IF(NH.GT.NI.AND.LGKWD.LE.-10.0)  WRITE(OUT,20482)
     1                  LGKWD
      IF(NSTOP.EQ.0)  GO TO 1000
      IF(TRIAL)  WRITE(OUT,20485)
C
C
C     SECTION FIVE.     CONSTANT EVALUATION.
C
C
  500 K = NK
      KOUNT = 0
      DO 505 II=1,NII
  505 XXLOG(II) = ALOG10(XX(II))
      DO 512 J=1,NJ
      CONST(J) = BETALG(J)
      IF(NUMF(J).EQ.0)  GO TO 512
      N = NUMF(J)
      DO 511 L=1,N
      II = KEY(K) - NI
      CONST(J) = CONST(J) + XXLOG(II)
  511 K = K - 1
  512 CONST(J) = 10.000D0 ** CONST(J)
C
C
C     SECTION SIX.     EVALUATION OF CALCULATED SPECIES
     1                 CONCENTRATIONS.
C
```

```
C
  600 K = 1
      DO 602 J=1,NJ
      SPECIE(J) = CONST(J)
      N = NUMT(J)
      IF(N.EQ.0)  GO TO 602
      DO 601 L=1,N
      I = KEY(K)
      SPECIE(J) = SPECIE(J) * X(I)
  601 K = K + 1
  602 CONTINUE
C
C
C     SECTION SEVEN.   EVALUATION OF CALCULATED TOTAL
                  CONCENTRATIONS.
C
C
  700 K = 1
      DO 701 I=1,NI
  701 TCALC(I) = X(I)
      DO 703 J=1,NJ
      N = NUMT(J)
      IF(N.EQ.0)  GO TO 703
      DO 702 L=1,N
      I = KEY(K)
      TCALC(I) = TCALC(I) + SPECIE(J)
  702 K = K + 1
  703 CONTINUE
      IF(.NOT.TRIAL)  GO TO 800
      L = MIN0(3,NI)
      WRITE(OUT,20701)  KOUNT, (TCALC(I), X(I), I=1,L)
C
C
C     SECTION EIGHT.     TEST FOR CONVERGENCE.
C
C
C
  800 KOUNT = KOUNT + 1
      IF(MOD(KOUNT,100).EQ.25)  TLIM = TLIM * 1.5
      L = 0
      DO 801 I=1,NI
      CRIT = TREAL(I) - TCALC(I)
      CRIT = ABS(CRIT)
      CRIT = CRIT - (TREAL(I) * TLIM)
      IF(CRIT.LE.0.0000)  L = L + 1
  801 CONTINUE
      IF(KOUNT.LT.NSTOP.AND.L.LT.NI)  GO TO 900
      IF(KOUNT.LT.NSTOP)  GO TO 1000
      IF(.NOT.TRIAL)  WRITE(OUT,20801)
      SCAN = .FALSE.
      GO TO 1000
C
C
C     SECTION NINE.     EVALUATION OF THE COMPONENTS NEW
                  FREE CONCENTRATIONS.
```

```
C
C
C
  900 DO 901 I=1,NI
  901 FDASH(I) = TREAL(I) / TCALC(I)
      IF(KOUNT.LT.4.OR.KOUNT.GT.49.OR.
     1   KOUNT.EQ.30.OR.KOUNT.EQ.40.OR.KOUNT.EQ.46)
                  GO TO 903
      IF(KOUNT.GT.14.AND.MOD(KOUNT,3).EQ.1)  GO TO 905
      DO 902 I=1,NI
      CRIT = FDASH(I)
  902 FDASH(I) = SQRT(CRIT)
      GO TO 905
  903 DO 904 I=1,NI
  904 FDASH(I) = FDASH(I) ** POWER(I)
      IF(KOUNT.LT.10.OR.MOD(KOUNT,50).EQ.0)  GO TO 915
  905 DO 906 I=1,NI
  906 X(I) = X(I) * FDASH(I)
      GO TO 600
C
C
C
  915 IF(SCAN.AND.NCYCLE.GT.1.AND.KOUNT.GT.1)  GO TO 905
      DO 919 M=1,NI
      IXA = 1
      POWER(M) = 1.000 / POWER(M)
      DEN = X(M)
      K = 1
      DO 918 J=1,NJ
      N = NUMT(J)
      IF(N.EQ.0)  GO TO 918
      KIJ = 0
      DO 916 L=1,N
      IF(KEY(K).EQ.M)  KIJ = KIJ + 1
  916 K = K + 1
      IF(KIJ.EQ.0)  GO TO 918
      IF(SPECIE(J)*100.0.GT.TCALC(M).AND.KIJ.GT.IXA)
                  IXA = KIJ
      FACTOR = 1.000000
      K = K - N
      DO 917 L=1,N
      I = KEY(K)
      FACTOR = FACTOR * FDASH(I)
  917 K = K + 1
      DEN = DEN + (FACTOR * SPECIE(J) * KIJ / FDASH(M))
  918 CONTINUE
      XOLD = FLOAT(IXA)
      IF(XOLD+0.1.LT.POWER(M))  POWER(M) = POWER(M) -
                  1.000
      IF(XOLD.GT.POWER(M))  POWER(M) = XOLD
      POWER(M) = 1.000 / POWER(M)
      XOLD = X(M)
      X(M) = XOLD * TREAL(M) / DEN
  919 FDASH(M) = X(M) / XOLD
      GO TO 600
```

```
C
C
C       SECTION TEN.      OUTPUT
C
C
 1000 IF(SCAN)  WRITE(OUT,21000)  NCYCLE
      IF(.NOT.SCAN)  WRITE(OUT,21001)
      WRITE(OUT,21002)
      WRITE(OUT,21003)
C
C
C
C       SUM TOTAL CONCENTRATIONS FOR SPECIES WITH FREE
                    CONCS. FIXED.
C
      DO 1001 II=1,NII
      I = NI + II
 1001 TCALC(I) = XX(II)
      K = NK
      DO 1003 J=1,NJ
      N = NUMF(J)
      IF(N.EQ.0)  GO TO 1003
      DO 1002 L=1,N
      II = KEY(K)
      TCALC(II) = TCALC(II) + SPECIE(J)
 1002 K = K - 1
 1003 CONTINUE
C
C       OUTPUT COMPONENT CONCENTRATIONS
C
 1004 IXA = NLINES - 10
      IF(SCAN)  IXA = IXA - 5
      KIJ = ((NIII - IXA) / (NLINES - 5)) + 2
      IF(NIII.LE.IXA)  KIJ = 1
      N = (NIII / KIJ) + 1
      IF(IXA.GE.N)  IXA = N
      IF(IXA.GE.N.OR.KIJ.EQ.1)  GO TO 1005
      N = ((NIII - IXA) / (KIJ - 1)) + 1
 1005 KIJ = IXA
      DO 1006 I=1,NI
      IF(I.LT.KIJ.OR.I.EQ.NI)  GO TO 1006
      KIJ = KIJ + N
      WRITE(OUT,21001)
      WRITE(OUT,21003)
 1006 WRITE(OUT,21004)  I, X(I), TREAL(I), TCALC(I),
                    NCOMP(I), CHECK(I),
     1  POWER(I)
      M = NI
      DO 1008 II=1,NII
      M = M + 1
      IF(M.LT.KIJ.OR.II.EQ.NII)  GO TO 1007
      KIJ = KIJ + N
      WRITE(OUT,21001)
      WRITE(OUT,21003)
 1007 XOLD = 0.0
```

```
      IF(NCOMP(M).EQ.LIT(12).OR.NCOMP(M).EQ.LIT(13))
     1             GO TO 1008
      IF(PMI(II).LE.0.000)  GO TO 1008
      XOLD = TCALC(M) / PMI(II)
      XOLD = ALOG10(XOLD)
 1008 WRITE(OUT,21005)  M, XX(II), TCALC(M), NCOMP(M),
     1             CHECK(M), XOLD
      IF(.NOT.SCAN)  GO TO 1010
      IF((NI+NII).LE.10) WRITE(OUT,20301)
      IF(NN.GT.NI)  WRITE(OUT,20481)  PH
C
C     OUTPUT SPECIES CONCENTRATIONS
C
 1010 IF(SUPRES)  GO TO 1028
      IF(NLINES.LT.50.OR.NIII+NJ.GT.NLINES-40)
     1             WRITE(OUT,20000)
      WRITE(OUT,20301)
      WRITE(OUT,21011)
      WRITE(OUT,21012)
      NJ = NJ + 5
      IXA = NLINES - 10
      KIJ = ((NJ - IXA) / (NLINES - 5)) + 2
      IF(NJ.LE.IXA)  KIJ = 1
      NSP1 = (NJ / KIJ) + 1
      IF(IXA.GT.NSP1)  IXA = NSP1
      IF(IXA.GE.NSP1.OR.KIJ.EQ.1)  GO TO 1011
      NSP1 = ((NJ - IXA) / (KIJ - 1)) + 1
 1011 KIJ = IXA
      NJ = NJ - 5
      NXP1 = KIJ + (3*NSP1) + 6
C
C
C
      NKT = 1
      NKF = NK
      DO 1027 J=1,NJ
      IF(J.LT.KIJ.OR.J.EQ.NJ)  GO TO 1020
      IF(KIJ.LT.NXP1)  KIJ = KIJ + 1
      KIJ = KIJ + NSP1
      WRITE(OUT,20000)
      WRITE(OUT,21001)
      WRITE(OUT,21012)
 1020 N = 1
      NN = NUMT(J)
      IF(NN.EQ.0)  GO TO 1023
      L = 0
 1021 K = KEY(NKT)
      M = 0
 1022 L = L + 1
      M = M + 1
      NKT = NKT + 1
      IF(L.LT.NN.AND.K.EQ.KEY(NKT))  GO TO 1022
      TITLE(N) = NCOMP(K)
      N = N + 1
      TITLE(N) = M
```

```
      N = N + 1
      IF(L.LT.NN)  GO TO 1021
      IF(NUMF(J).EQ.0)  GO TO 1026
 1023 L = 0
      NN = NUMF(J)
 1024 K = KEY(NKF)
      M = 0
 1025 L = L + 1
      M = M + 1
      NKF = NKF - 1
      IF(L.LT.NN.AND.K.EQ.KEY(NKF))  GO TO 1025
      TITLE(N) = NCOMP(K)
      N = N + 1
      TITLE(N) = M
      N = N + 1
      IF(L.LT.NN)  GO TO 1024
 1026 NN = N - 1
 1027 WRITE(OUT,21021) J, SPECIE(J), BETALG(J),
                  (TITLE(N), N=1,NN)
 1028 WRITE(OUT,21022)  KOUNT
      IF(KOUNT.GE.NSTOP)  WRITE(OUT,21023)
      WRITE(OUT,21024)  TLIM
C
C
C     SECTION ELEVEN.     THE COMPONENT MONITOR.
C
C
 1100 IF(.NOT.MONIT)  GO TO 1200
      IF(MONALL)  NMON = NIII
      LAYBY = 1
      IF(PUNCH)  LAYBY = 2
      DO 1136 KIJ=LAYBY,NMON
      IF(PUNCH)  WRITE(CARD,31100)  LIT(1)
      IXA = NLINES - 10
      NN = 0
      M = KIJ
      IF(.NOT.MONALL)  M = IMON(KIJ)
      IML = M - NI
      IF(M.LE.NI)  XOLD = X(M)
      IF(M.GT.NI) XOLD = XX(IML)
      DEN = XOLD * 100.0 / TCALC(M)
      WRITE(OUT,21101)  NCOMP(M), XOLD, DEN
C
C     LOAD THE SPECIES WHICH CONTAIN THIS MONITORED
                  COMPONENT.
C
      IF(M.GT.NI)  GO TO 1110
      IF(PUNCH)  WRITE(CARD,31100)  NCOMP(M), TCALC(M),
                  X(M)
      NKT = 1
      DO 1104 J=1,NJ
      N = NUMT(J)
      IF(N.EQ.0)  GO TO 1104
      K = NKT
      DEN = 0.00
```

```
      DO 1101 L=1,N
      IF(KEY(K).EQ.M)  DEN = DEN + 1.0
 1101 K= K + 1
      IF(DEN.LT.0.1)  GO TO 1103
 1102 NN = NN + 1
      JSP(NN) = J
      SPC(NN) = SPECIE(J) * DEN
 1103 NKT = NKT + N
 1104 CONTINUE
      GO TO 1120
 1110 NKF = NK
      J = M - NI
      IF(PUNCH)  WRITE(CARD,31100)  NCOMP(M), TCALC(M),
                  XX(J)
      DO 1113 J=1,NJ
      N = NUMF(J)
      IF(N.EQ.0)  GO TO 1113
      K = NKF
      DEN = 0.00
      DO 1111 L=1,N
      IF(KEY(K).EQ.M)  DEN = DEN + 1.0
 1111 K = K - 1
      IF(DEN.LT.0.1)  GO TO 1113
 1112 NN = NN + 1
      JSP(NN) = J
      SPC(NN) = SPECIE(J) * DEN
 1113 NKF = NKF - N
C
C     SORT THEM.
C
 1120 CALL SORT(SPC,JSP,NN)
      WRITE(OUT,21012)
C
C    SET UP THE DIRECTORY ARRAYS.
C
      NKT = 1
      NKF = NK
      DO 1121 J=1,NJ
      NST(J) = NKT
      NSF(J) = NKF
      NKT = NKT + NUMT(J)
 1121 NKF = NKF - NUMF(J)
C
C     OUTPUT EACH SPECIES IN TURN.
C
      IF(IXA.GT.NN)  IXA = NN
      DO 1136 I=1,IXA
      J = JSP(I)
      N = 1
      NN = NUMT(J)
      IF(NN.EQ.0)  GO TO 1132
      L = 0
      NKT = NST(J)
 1130 K = KEY(NKT)
      NXP1 = 0
```

```
 1131 L = L + 1
      NXP1 = NXP1 + 1
      NKT = NKT + 1
      IF(L.LT.NN.AND.K.EQ.KEY(NKT))  GO TO 1131
      TITLE(N) = NCOMP(K)
      N = N + 1
      TITLE(N) = NXP1
      N = N + 1
      IF(L.LT.NN)  GO TO 1130
      IF(NUMF(J).EQ.0)  GO TO 1135
 1132 L = 0
      NN = NUMF(J)
      NKF = NSF(J)
 1133 K = KEY(NKF)
      NXP1 = 0
 1134 L = L + 1
      NXP1 = NXP1 + 1
      NKF = NKF + 1
      IF(L.LT.NN.AND.K.EQ.KEY(NKF))  GO TO 1134
      TITLE(N) = NCOMP(K)
      N = N + 1
      TITLE(N) = NXP1
      N = N + 1
      IF(L.LT.NN)  GO TO 1133
 1135 NN = N - 1
      IF(PUNCH)  WRITE(CARD,31101)  SPECIE(J), (TITLE(N),
                 N=1,NN)
      DEN = (SPC(I) * 100.0)  /  TCALC(M)
 1136 WRITE(OUT,21131) J, SPECIE(J), DEN, BETALG(J),
                 (TITLE(N), N=1,NN)
C
C
C     SECTION TWELVE.     THE SCAN MECHANISM.
C
C
 1200 IF(.NOT.SCAN)  GO TO 9999
C
C
      NCYCLE = NCYCLE + 1
      TLIM = 1.5E-6
      IF(ISCN.GT.NI)  GO TO 1201
      IF(MULTIP)  TREAL(ISCN) = TREAL(ISCN) * SCNINC
      IF(.NOT.MULTIP)  TREAL(ISCN) = TREAL(ISCN) + SCNINC
      IF(TREAL(ISCN).GT.SCNMAX)  GO TO 9999
      IF(NSIMUL.GT.0)  GO TO 1210
      IF(MONIT)  GO TO 500
      GO TO 600
 1201 II = ISCN - NI
      IF(MULTIP)  XX(II) = XX(II) * SCNINC
      IF(.NOT.MULTIP)  XX(II) = XX(II) + SCNINC
      IF(NH.NE.ISCN) GO TO 1202
      NN = NH - NI
      PH = XX(NN)
      PH = -1.000 * ALOG10(PH)
      IF (NOH .EQ. 0) GO TO 1202
```

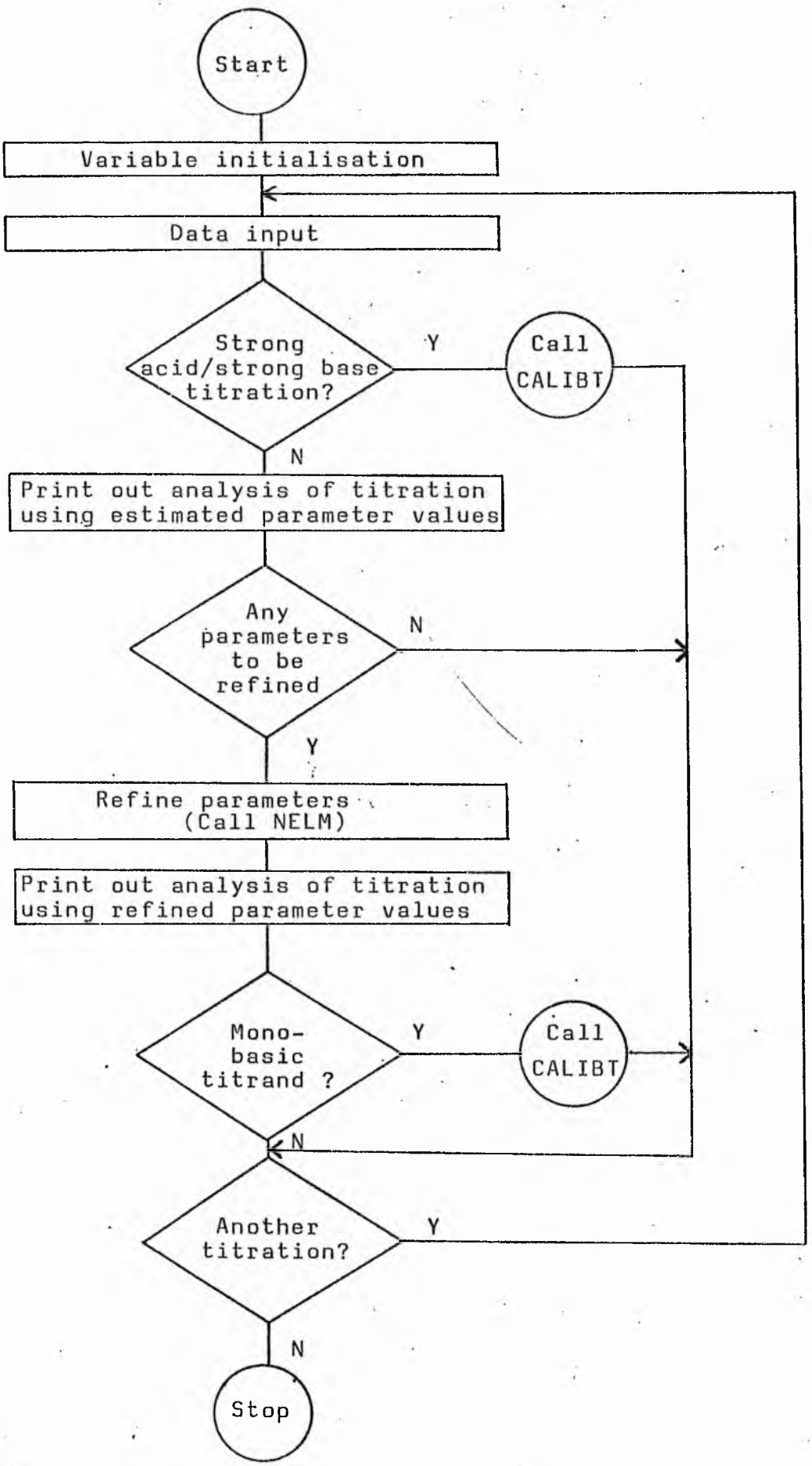
```
      NN = NOH - NI
      XX(NN) = 10.000 ** LGKWD  /  XX(II)
 1202 IF(XX(II).GT.SCNMAX)  GO TO 9999
      IF(NSIMUL.EQ.0)  GO TO 500
C
 1210 II = -1
      DO 1212 K=1,NSIMUL
      I = SIMUL(K)
      IF(I.GT.NI)  GO TO 1211
      IF(MULTIP)  TREAL(I) = TREAL(I) * SCNINC
      IF(.NOT.MULTIP)  TREAL(I) = TREAL(I) + SCNINC
      GO TO 1212
 1211 IF(II.EQ.NOH)  GO TO 1212
      II = I - NI
      IF(MULTIP)  XX(II) = XX(II) * SCNINC
      IF(.NOT.MULTIP)  XX(II) = XX(II) + SCNINC
 1212 CONTINUE
      IF(.NOT.MONIT.AND.II.EQ.-1)  GO TO 600
      GO TO 500
C
C
C     THE END.
C
C
 9999 WRITE(OUT,99999)
      STOP
      END
```

```
      SUBROUTINE SORT(ARRAY,NARRAY,N)
      DIMENSION  ARRAY(N), NARRAY(N)
      REAL  LAYBY
      INTEGER OLD
      M = 2
      DO 1 J=1,N
      M = M + M
      IF(M.GT.N)  GO TO 2
    1 CONTINUE
    2 M = M - 1
    3 M = (M - 1) / 2
    4 NN = N - M
      DO 7 I=1,NN
      OLD = I + M
      LAYBY = ARRAY(OLD)
      NLAYBY = NARRAY(OLD)
      DO 5 J=1,I,M
      NEW = OLD - M
      IF(LAYBY.LE.ARRAY(NEW))  GO TO 6
      ARRAY(OLD) = ARRAY(NEW)
      NARRAY(OLD)  = NARRAY(NEW)
      OLD = NEW
    5 CONTINUE
    6 ARRAY(OLD) = LAYBY
      NARRAY(OLD) = NLAYBY
    7 CONTINUE
      IF(M.GT.1)  GO TO 3
      RETURN
      END
```

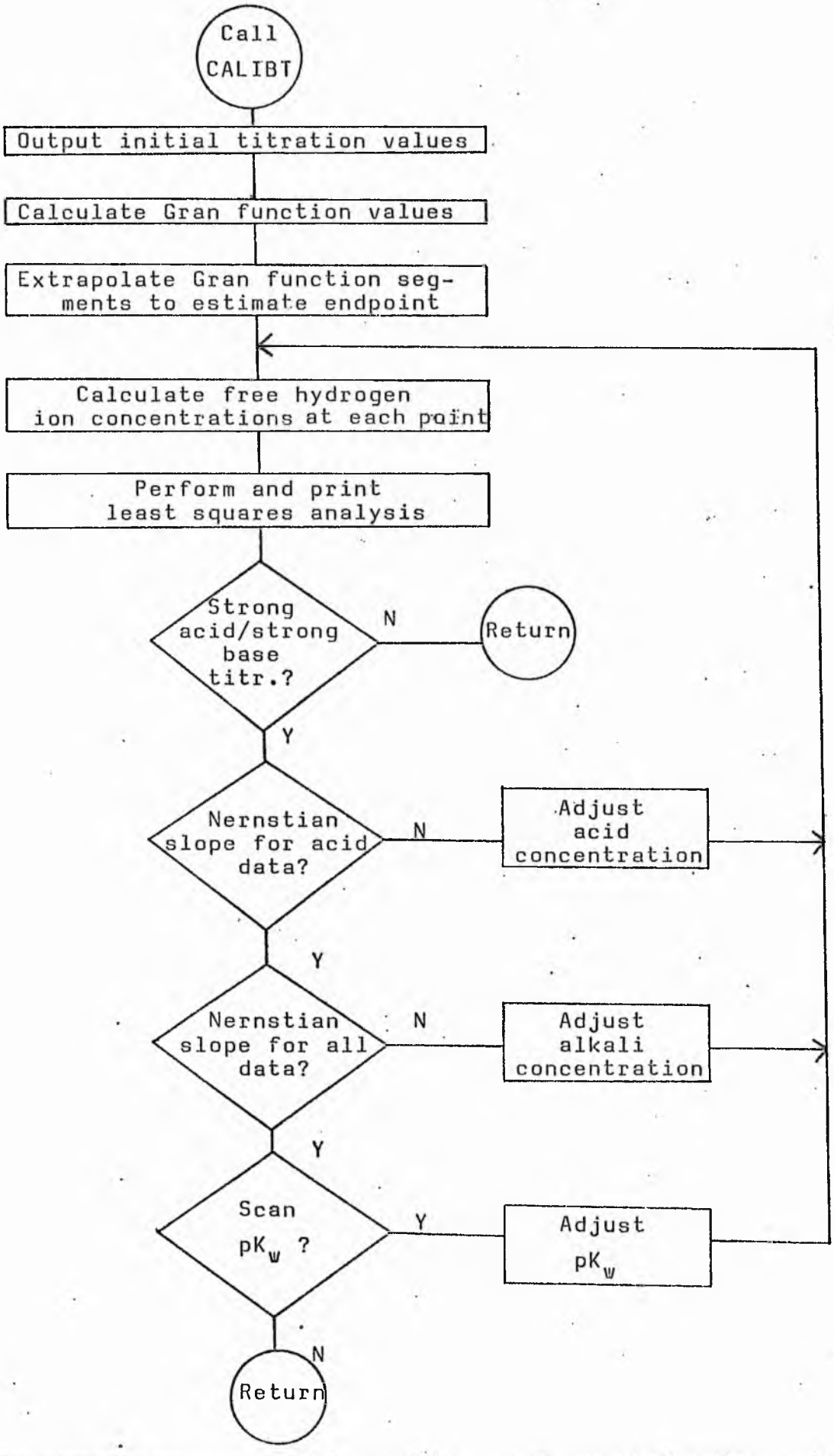
APPENDIX A4

FORTRAN listing of program MAGEC

FLOW DIAGRAM FOR PROGRAM MAGEC



FLOW DIAGRAM FOR PROGRAM MAGEC (CONTD)



```
C     ************************************************************
                ************
C
C
C                          PROGRAM MAGEC.
C                          --------------
C
C
C
C                        MULTIPLE ANALYSIS
C                      OF TITRATION DATA FOR
C                   GLASS ELECTRODE CALIBRATION.
C
C
C
C     ************************************************************
                ************
C
C
C
C     THE PROGRAM ANALYSES POTENTIOMETRIC DATA FROM
                TITRATIONS IN WHICH
C     THE TITRAND AND THE TITRANT ARE (1) A STRONG ACID
                OR A STRONG
C     BASE, (2) A LIGAND OR (3) A COMBINATION OF (1) AND
                (2).
C
C     ESTIMATES OF THE CONCENTRATIONS, THE EQUILIBRIUM
                CONSTANTS AND
C     THE PARAMETERS FOR THE NERNSTIAN EQUATION OF THE
                GLASS ELECTRODE
C     RESPONSE TO HYDROGEN ION CONCENTRATION CAN BE
                REFINED.   THE USER
C     SPECIFIES WHICH ARE TO BE SIMULTANEOUSLY OPTIMISED.
                  UPPER AND
C     LOWER LIMITS CAN BE IMPOSED ON ALL PARAMETERS BEING
                REFINED.
C
C     THE OPTIMISATION IS PERFORMED BY SUBROUTINE NELM
                AND GENERALLY,
C     THE EQUILIBRIUM CONCENTRATIONS ARE EVALUATED BY
                SUBROUTINE ML AND
C     SUBROUTINE FUNCT.
C
C     SUBPROGRAM CALIBT ONLY ACCEPTS DATA PERTAINING TO
                MONOBASIC
C     REACTANTS.   IT IS MAINLY USED TO ANALYSE STRONG
                ACID VERSUS
C     STRONG BASE TITRATIONS.   THE PARAMETERS OF THE
                NERNSTIAN
C     EQUATION ARE FOUND BY LINEAR LEAST SQUARES BEST FIT.
C     IN ADDITION IT PERFORMS GRAN PLOT CALCULATIONS,
                SCANS VALUES OF
C     PKW AND PK1 AND ADJUSTS THE CONCENTRATIONS OF
                REACTANTS IF THIS
```

```
C      IMPROVES THE AGREEMENT BETWEEN THE THEORETICAL AND
                  THE OBSERVED
C      SLOPE FOR THE GLASS ELECTRODE RESPONSE.
C
C
C
C      THE PROGRAM WAS DEVELOPED AT THE UNIVERSITY OF
                  WALES INSTITUTE
C      OF SCIENCE AND TECHNOLOGY IN 1978. IT IS WRITTEN IN
                  FORTRAN IV.
C
C
C
C      ************************************************************
                  **************
C
C
C
       INTEGER  TITLE, LIT(15)
       DIMENSION  X(7), Y(7), H(7), XS(7), FP(8), ID(7)
       INTEGER  OUT, REFINE
       REAL LVESSL, LBURET
       LOGICAL TITEND
       COMMON /ONE/  PK(7,3), VZERO(3), HVESSL(3),
                  LVESSL(3), HBURET(3),
      *    LBURET(3), EZERO(3), SLOPE(3), REFINE(15), NDPV,
                  NDPB
       COMMON /TWO/ CI(7),CX(2),TT(2),HX(2),TOLC(2),DT(2),
                  DDT(2,2)
       COMMON /THREE/  V(100), E(100), NP
       COMMON /FOUR/  BETA(7), ARRAY(3), TOL, NCONST,
                  NBETAH, JQR(2,7)
       COMMON /FIVE/  IN, OUT, IFAIL, JFAIL, AL10, KOUNT
       COMMON /SIX/  TITLE(20), P(63)
C
C
       DATA  LIT /'PKW','PK1','PK2','PK3','PK4','PK5',
                  'PK6',
      *    'VZO','H+V','LGV','H+B','LGB','EZO','SLP','   '/
C
C
C
10200  FORMAT(20A4)
10231  FORMAT(8G10.3)
10205  FORMAT(A3,2X,I1,1X,I1,2X,3G10.3)
20000  FORMAT('1')
20001  FORMAT(//'0',1H*,3X,1H*,6X,3(1H*),7X,4(1H*),5X,
                  5(1H*),6X,
      *    4(1H*),/' ',2(2(1H*),1X),4X,1H*,3X,1H*,5X,3(1H*,
                  9X),
      *    /' ',1H*,1X,1H*,1X,1H*,5X,5(1H*),5X,1H*,1X,
                  3(1H*),
      *    5X,4(1H*),6X,1H*,/' ',3(1H*,3X,1H*,5X),1H*,9X,
                  1H*,
```

```
     *    /' ',2(1H*,3X,1H*,5X),1X,4(1H*),5X,5(1H*),6X,
                4(1H*),
     *    /' ',45(1H-),////)
20200 FORMAT(///'0',30X,20A4)
20203 FORMAT(////'0',4X,'INPUT VALUES FOR THE TITRATION
                PARAMETERS ARE:'
     *    ,///'0',4X,'IDENTIFIER',4X,'REFINE
                KEY',7X,'NDP',10X,'VALUE',
     *    26X,'LOWER AND UPPER LIMITS',//)
20204 FORMAT('0',8X,A3,12X,I1,13X,F17.3,17X,2F15.3)
20205 FORMAT('0',8X,A3,12X,I1,13X,1PE17.4,17X,2E15.4)
20206 FORMAT('0',8X,A3,12X,I1,12X,I1,1PE17.4,20X,2E15.4)
20208 FORMAT('+',89X,'N/A')
20234 FORMAT(///'0DIMENSION LIMITS OF TITRATION DATA
                ARRAYS EXCEEDED')
20250 FORMAT(///'0','SEQUENCE ERROR DETECTED IN THE DATA')
20260 FORMAT(///'0','ERROR IN THE LIMITS FOR REFINEMENT')
20270 FORMAT(///'0','ACID DISSOCIATION CONSTANT ERROR')
20290 FORMAT('0','EXECUTION TERMINATED.',///)
20302 FORMAT(///'0',4X,'V(OBS.) V(CALC.) EMF(OBS.)
     ,          EMF(CALC.)',
     2  7X,'RESIDUALS',12X,'PH',9X,'FL',8X,
     2  'EZO(CALC.)  SLP(CALC.)',///)
20305 FORMAT(////'0',2X,'REFINED VALUES FOR THE
                PARAMETERS ARE NOW:',///
     1 '0',2X,'OBJ. FUNCT.',5X,A4,6(8X,A4))
20306 FORMAT('+',102X,'OPTIMIZATION RECORD',//)
20310 FORMAT('0',1P8E12.3)
20311 FORMAT('+',102X,7(1H*),3X,'CONVERGED',//)
C
C
C     SECTION ONE;   INITIALISATION.
C
C
      IN = 5
      OUT = 6
      IFAIL = 0
      JFAIL = 0
      EPS = 1.0E-6
      TOL = 1.0E-4
      AL10 = ALOG(10.00)
      TITEND = .FALSE.
C
C
C     SECTION TWO;   DATA INPUT
C
C
  200 WRITE(OUT,20000)
      READ(IN,10200,END=299)  TITLE
      WRITE(OUT,20001)
      WRITE(OUT,20200)  TITLE
      WRITE(OUT,20203)
      M = 1
      DO 207 I=1,7
      READ(IN,10205)  KEY, IVAL, JVAL, (PK(I,J), J=1,3)
```

```
      N = 1
      IF(IVAL.EQ.2)  N = 3
      WRITE(OUT,20204)  KEY, IVAL, (PK(I,J), J=1,N)
      IF(N.EQ.1)  WRITE(OUT,20208)
      IF(KEY.EQ.LIT(8))  GO TO 210
      IF(KEY.NE.LIT(I))  GO TO 250
      DO 205 L=1,3
  205 ARRAY(L) = PK(I,L)
      IF(IVAL.GT.0)  CALL SETUP(ID,LIT(I),M,X,H,IVAL,
                    ARRAY)
      IF(I.LE.2)  GO TO 207
      IF(PK(I,1).LT.PK(I-1,1))  GO TO 270
  207 REFINE(I+7) = IVAL
      READ(IN,10205)  KEY, IVAL, JVAL, VZERO
      IF(KEY.NE.LIT(8))  GO TO 290
      IF(IVAL.EQ.2.AND.(PK(I,2).GT.PK(I,1).OR.PK(I,
                    3).LT.PK(I,1)))
     *   GO TO 260
      GO TO 215
C
C     REFINE(1)   =  VZERO
C     REFINE(2)   =  HVESSL
C     REFINE(3)   =  LVESSL
C     REFINE(4)   =  HBURET
C     REFINE(5)   =  LBURET
C     REFINE(6)   =  EZERO
C     REFINE(7)   =  SLOPE
C     REFINE(8)   =  PKW
C     REFINE(9) TO REFINE(14)  =  PK1 TO PK6
C
  210 NCONST = I - 1
      DO 212 J=1,3
  212 VZERO(J) = PK(I,J)
  215 REFINE(1) = IVAL
      IF(REFINE(1).EQ.2.AND.(VZERO(2).GT.VZERO(1)
                    .OR.VZERO(3)
     *   .LT.VZERO(1)))  GO TO 260
      IF(REFINE(1).GT.0)  CALL SETUP(ID,LIT(8),M,X,H,
                    REFINE(1),VZERO)
      READ(IN,10205)  KEY, REFINE(2), JVAL, HVESSL
      N = 1
      IF(REFINE(2).EQ.2)  N = 3
      WRITE(OUT,20205)  KEY, REFINE(2), (HVESSL(J), J=1,N)
      IF(N.EQ.1)  WRITE(OUT,20208)
      IF(KEY.NE.LIT(9))  GO TO 250
      IF(REFINE(2).EQ.2.AND.(HVESSL(2).GT.HVESSL(1)
                    .OR.HVESSL(3)
     *   .LT.HVESSL(1)))  GO TO 260
      IF(REFINE(2).GT.0)  CALL SETUP(ID,LIT(9),M,X,H,
                    REFINE(2),HVESSL)
      READ(IN,10205)  KEY, REFINE(3), NDPV, LVESSL
      N = 1
      IF(REFINE(3).EQ.2)  N = 3
      WRITE(OUT,20206)  KEY, REFINE(3), NDPV, (LVESSL(J),
                    J=1,N)
```

```
      IF(N.EQ.1)  WRITE(OUT,20208)
      IF(KEY.NE.LIT(10))  GO TO 250
      IF(REFINE(3).EQ.2.AND.(LVESSL(2).GT.LVESSL(1)
                .OR.LVESSL(3)
     *    .LT.LVESSL(1)))  GO TO 260
      IF(REFINE(3).GT.0)  CALL SETUP(ID,LIT(10),M,X,H,
                REFINE(3),LVESSL)
      READ(IN,10205)  KEY, REFINE(4), JVAL, HBURET
      N = 1
      IF(REFINE(4).EQ.2)  N = 3
      WRITE(OUT,20205)  KEY, REFINE(4), (HBURET(J), J=1,N)
      IF(N.EQ.1)  WRITE(OUT,20208)
      IF(KEY.NE.LIT(11))  GO TO 250
      IF(REFINE(4).EQ.2.AND.(HBURET(2).GT.HBURET(1)
                .OR.HBURET(3)
     *    .LT.HBURET(1)))  GO TO 260
      IF(REFINE(4).GT.0)  CALL SETUP(ID,LIT(11),M,X,H,
                REFINE(4),HBURET)
      READ(IN,10205)  KEY, REFINE(5), NDPB, LBURET
      N = 1
      IF(REFINE(5).EQ.2)  N = 3
      WRITE(OUT,20206)  KEY, REFINE(5), NDPB, (LBURET(J),
                J=1,N)
      IF(N.EQ.1)  WRITE(OUT,20208)
      IF(KEY.NE.LIT(12))  GO TO 250
      IF(REFINE(5).EQ.2.AND.(LBURET(2).GT.LBURET(1)
                .OR.LBURET(3)
     *    .LT.LBURET(1)))  GO TO 260
      IF(REFINE(5).GT.0)  CALL SETUP(ID,LIT(12),M,X,H,
                REFINE(5),LBURET)
      READ(IN,10205)  KEY, REFINE(6), JVAL, EZERO
      N = 1
      IF(REFINE(6).EQ.2)  N = 3
      WRITE(OUT,20204)  KEY, REFINE(6), (EZERO(J), J=1,N)
      IF(N.EQ.1)  WRITE(OUT,20208)
      IF(KEY.NE.LIT(13))  GO TO 250
      IF(REFINE(6).EQ.2.AND.(EZERO(2).GT.EZERO(1)
                .OR.EZERO(3)
     *    .LT.EZERO(1)))  GO TO 260
      IF(REFINE(6).GT.0)  CALL SETUP(ID,LIT(13),M,X,H,
                REFINE(6),EZERO)
      READ(IN,10205)  KEY, REFINE(7), JVAL, SLOPE
      N = 1
      IF(REFINE(7).EQ.2)  N = 3
      WRITE(OUT,20204)  KEY, REFINE(7), (SLOPE(J), J=1,N)
      IF(N.EQ.1)  WRITE(OUT,20208)
      IF(KEY.NE.LIT(14))  GO TO 250
      IF(REFINE(7).EQ.2.AND.(SLOPE(2).GT.SLOPE(1)
                .OR.SLOPE(3)
     *    .LT.SLOPE(1)))  GO TO 260
      IF(REFINE(7).GT.0)  CALL SETUP(ID,LIT(14),M,X,H,
                REFINE(7),SLOPE)
C
      DO 231 I=1,100.
      READ(IN,10231,END=235)  V(I), E(I)
```

```
      IF(V(I).LT.0.000)  GO TO 239
  231 CONTINUE
      I = 101
      READ(IN,10205,END=235)  KEY
      WRITE(OUT,20234)
      GO TO 290
  235 TITEND = .TRUE.
  239 NP = I - 1
      GO TO 300
C
  250 WRITE(OUT,20250)
      GO TO 290
  260 WRITE(OUT,20260)
      GO TO 290
  270 WRITE(OUT,20270)
  290 WRITE(OUT,20290)
  299 STOP
C
C
C     SECTION THREE;   REFINEMENT BY SUBROUTINE NELM.
C
C
  300 NBETAH = NCONST - 1
      M = M - 1
      IF(NBETAH.LT.1)  GO TO 400
      DO 301 I=1,NBETAH
      JQR(1,I) = I
  301 JQR(2,I) = 1
      JQR(1,NCONST) = -1
      JQR(2,NCONST) = 0
C     IF(M-1)  350, 320, 340
C 320 KOUNT = -50
C     X(1) = Y(1) + H(1)
C     WRITE(OUT,20302)
C     CALL CALC(M,X,F,&325)
C 325 X(1) = Y(1) - H(1)
C     WRITE(OUT,20302)
C     CALL CALC(M,X,F,&327)
C 327 X(1) = Y(1)
C     GO TO 350
      IF(M.EQ.0)  GO TO 350
  340 KOUNT = -25
      WRITE(OUT,20302)
      CALL CALC(0,X,F,&341)
  341 KOUNT = 0
      WRITE(OUT,20305)  (ID(I), I=1,M)
      WRITE(OUT,20306)
      DO 344 I=1,M
  344 Y(I) = X(I)
      IF(M.LT.7)  Y(M+1) = SLOPE(1)
      M1 = M + 1
      MP = M * (M + 2)
      CALL NELM(X,F,EPS,P,FP,XS,H,0,M,M1,MP)
      IF(KOUNT.LE.M)  GO TO 355
      WRITE(OUT,20310)  F, (X(I), I=1,M)
```

```
      IF(IFAIL.EQ.0)      WRITE(OUT,20311)
      IFAIL = 0
C
  350 KOUNT = -100
      WRITE(OUT,20302)
      CALL CALC(M,X,F,&355)
C     RESET ORIGINAL PARAMETER VALUES.
  355 REFINE(7) = 2
      SLOPE(3) = SLOPE(1) - ABS(SLOPE(1)) / 10.00
      SLOPE(2) = SLOPE(1)
  360 CALL CALC(M,Y,F,&400)
C
C
C     SECTION FOUR
C
C
  400 IF(NBETAH.GT.1.OR.ABS(LBURET(1)).GT.1.0E-8)
                  GO TO 999
      IF(LVESSL(1).GT.1.0E-8.AND.HBURET(1)
                  .LE.0..AND.NDPV.NE.1) GOTO 999
      IF(LVESSL(1).GT.1.0E-8.AND.HBURET(1)
                  .GT.0..AND.NDPV.NE.0) GOTO 999
      PKWMIN = PK(1,1)
      PKWMAX = 0.000
      BLGMIN = PK(2,1)
      BLGMAX = 0.000
      IF(REFINE(8).NE.2)  GO TO 401
      PKWMIN = PK(1,2)
      PKWMAX = PK(1,3)
      GO TO 450
  401 IF(REFINE(9).NE.2)  GO TO 450
      BLGMIN = PK(2,2)
      BLGMAX = PK(2,3)
  450 CALL CALIBT(VZERO(1),EZERO(1),SLOPE(1),HVESSL(1),
                  LVESSL(1),
C
     *  HBURET(1),PKWMIN,PKWMAX,BLGMIN,BLGMAX)
C
C
  999 IF(.NOT.TITEND)  GO TO 200
      STOP
      END
```

```
      SUBROUTINE SETUP(ID,LIT,M,X,H,IREF,ARRAY)
      DIMENSION  X(7), H(7), ARRAY(3), ID(7)
      INTEGER OUT
      COMMON /FIVE/  IN, OUT, IFAIL, JFAIL, AL10, KOUNT
      IF(M.GE.8)  GO TO 20
      IF(IREF.EQ.2)  GO TO 10
      IXA = 1
      XA = ARRAY(1) / 12.000
      IF(XA.LT.0.0000)  IXA = -1
      ARRAY(2) = ARRAY(1) - XA * IXA
      ARRAY(3) = ARRAY(1) + XA * IXA
   10 H(M) = (ARRAY(2) + (ARRAY(3) - ARRAY(2)) / 10.0) -
                  ARRAY(1)
```

```
      X(M) = ARRAY(1)
      ID(M) = LIT
      M = M + 1
      RETURN
   20 WRITE(OUT,99)
   99 FORMAT(//'0','ATTEMPT TO REFINE TOO MANY
                  PARAMETERS',/' ',
     1 'ERROR TERMINATION',///)
      STOP
      END
```

```
      SUBROUTINE NELM(X,F,EPS,P,FP,XS,H,IX,N,N1,NP)
      DIMENSION  X(N), P(NP), FP(N1), H(N), XS(N)
      ISH = 1
      IS = 0
      NN = N * (N + 1)
      CALL CALC(N,X,F,&100)
      FP(1) = F
      IF(IX.NE.0)  GO TO 2
      DO 1 I=1,N
      K = I
      DO 1 J=1,N1
      P(K) = X(I)
      IF(I-J+1.NE.0)  GO TO 1
      P(K) = X(I) + H(I)
    1 K = K + N
    2 K = 1 + N
      DO 3 I=2,N1
      DO 4 J=1,N
      X(J) = P(K)
    4 K = K + 1
      CALL CALC(N,X,F,&100)
    3 FP(I) = F
      IF(FP(1)-FP(2).GT.0.0)  GO TO 5
      IH = 2
      IL = 1
      GO TO 6
    5 IH = 1
      IL = 2
    6 DO 7 I=3,N1
      IF(FP(I)-FP(IH).GT.0.0)  GO TO 8
      IF(FP(I)-FP(IL).GE.0.0)  GO TO 7
      IL = I
      GO TO 7
    8 IH = I
    7 CONTINUE
      XN = N
   50 K1 = NN
      DO 9 I=1,N
      K = I
      S = 0.0
      DO 10 J=1,N1
      IF(J-IH.EQ.0)  GO TO 10
      S = S + P(K)
   10 K = K + N
      K1 = K1 + 1
```

```
     9 P(K1) = S / XN
       K = NN + 1
       DO 11 I=1,N
       X(I) = P(K)
    11 K = K + 1
       CALL CALC(N,X,F0,&100)
       WRITE(6,12341)  IL, IH, IS
12341 FORMAT('+',100X,3I3,3X,'TEST')
       S = 0.0
       DO 12 I=1,N1
    12 S = S + (FP(I) - F0) ** 2
       S = S / XN
       IF(S-EPS.LE.0.00)  GO TO 100
       IF((IH-1).EQ.0)  GO TO 13
       IS = 1
       GO TO 14
    13 IS = 2
    14 DO 15 I=1,N1
       IF(I-IH.EQ.0)  GO TO 15
       IF(FP(I)-FP(IS).LE.0.0)  GO TO 15
       IS = I
    15 CONTINUE
C**** REFLECTION
       K = (IH - 1) * N + 1
       K0 = NN + 1
       DO 16 I=1,N
       X(I) = 2.0 * P(K0) - P(K)
       K = K + 1
    16 K0 = K0 + 1
       K = K - N
       CALL CALC(N,X,F,&100)
       WRITE(6,12342)  IL, IH, IS
12342 FORMAT('+',100X,3I3,3X,'REFLECTION')
       IF(F-FP(IL).GE.0.0)  GO TO 20
C**** EXPANSION
       K0 = NN + 1
       DO 17 I=1,N
       XS(I) = 2.0 * X(I) - P(K0)
    17 K0 = K0 + 1
       CALL CALC(N,XS,FS,&100)
       WRITE(6,12343)  IL, IH, IS
12343 FORMAT('+',100X,3I3,3X,'EXPANSION')
       IF(FS-FP(IL).GE.0.0)  GO TO 18
       DO 19 I=1,N
       P(K) = XS(I)
    19 K = K + 1
       FP(IH) = FS
       IL = IH
       IH = IS
       GO TO 50
    18 IL = IH
       IH = IS
       FP(IL) = F
    21 DO 22 I=1,N
       P(K) = X(I)
```

```
   22 K= K + 1
      GO TO 50
   20 IF(F-FP(IS).GE.0.0)  GO TO 23
      FP(IH) = F
      IH = IS
      GO TO 21
   23 IF(F-FP(IH).GE.0.0)  GO TO 25
      DO 24 I=1,N
      P(K) = X(I)
   24 K = K + 1
      FP(IH) = F
C**** CONTRACTION
      K = K - N
   25 K0 = NN + 1
      DO 26 I=1,N
      XS(I) = 0.5 * (P(K) + P(K0))
      K = K + 1
   26 K0 = K0 + 1
      K = K - N
      CALL CALC(N,XS,FS,&100)
      WRITE(6,12344)  IL, IH, IS
12344 FORMAT('+',100X,3I3,3X,'CONTRACTION')
      IF(FS-FP(IH).GE.0.0)  GO TO 40
      DO 27 I=1,N
      P(K) = XS(I)
   27 K = K + 1
      FP(IH) = FS
      IF(FP(1)-FP(2).GT.0.0)  GO TO 28
      IH = 2
      GO TO 29
   28 IH = 1
   29 DO 31 I=3,N1
      IF(FP(I)-FP(IH).LE.0.0)  GO TO 31
      IH = I
   31 CONTINUE
      GO TO 50
   40 FP(1) = FP(IL)
      KKK = MOD(ISH,10)
      ISH = ISH + 1
      IF((IL-1).EQ.0)  GO TO 43
      K = (IL - 1) * N
      DO 41 I=1,N
      K = K + 1
      X(I) = P(K)
      P(K) = P(I)
   41 P(I) = X(I)
      IL = 1
   43 K = N
      DO 42 I=2,N1
      DO 42 J=1,N
      K = K + 1
      P(K) = 0.5 * (P(K) + P(J))
      ISH = ISH + 1
   42 CONTINUE
      GO TO 2
```

```
  100 IL = 1
      DO 101 I=2,N1
      IF(FP(I)-FP(IL).GE.0.00)  GO TO 101
      IL = I
  101 CONTINUE
C     WRITE(6,2349)  IL, F0, (FP(I), I=1,N1)
C2349 FORMAT('0','NELM EXIT',I5,1P7E15.4,//)
      F = F0
      IF(F.LT.FP(IL))  RETURN
      K = (IL - 1) * N
      DO 102 I=1,N
      K = K + 1
      X(I) = P(K)
  102 CONTINUE
      F = FP(IL)
      RETURN
      END
```

```
      SUBROUTINE CALC(M,X,F,*)
      INTEGER  OUT, REFINE
      REAL LVESSL, LBURET
      COMMON /ONE/  PK(7,3), VZERO(3), HVESSL(3),
                    LVESSL(3), HBURET(3),
     *    LBURET(3), EZERO(3), SLOPE(3), REFINE(15), NDPV,
                    NDPB
      COMMON /TWO/ CI(7),CX(2),TT(2),HX(2),TOLC(2),DT(2),
                    DDT(2,2)
      COMMON /THREE/  V(100), E(100), NP
      COMMON /FOUR/  BETA(7), ARRAY(3), TOL, NCONST,
                    NBETAH, JQR(2,7)
      COMMON /FIVE/  IN, OUT, IFAIL, JFAIL, AL10, KOUNT
      DIMENSION X(M)
C
C
      IF(M.EQ.0)  GO TO 20
      KOUNT = KOUNT + 1
      IF(KOUNT.GE.M*50+80)  GO TO 60
C
C     LOAD THE PARAMETERS BEING REFINED.
C
      I = 0
      J = 7
      DO 15 K=1,NCONST
      J = J + 1
      IF(REFINE(J).LT.1)  GO TO 15
      DO 10 L=1,3
   10 ARRAY(L) = PK(K,L)
      CALL LOAD(&50,F,I,M,X,REFINE(J),ARRAY)
      PK(K,1) = ARRAY(1)
   15 CONTINUE
      IF(REFINE(1).GT.0)  CALL LOAD(&56,F,I,M,X,REFINE(1),
                    VZERO)
      IF(REFINE(2).GT.0)  CALL LOAD(&56,F,I,M,X,REFINE(2),
                    HVESSL)
      IF(REFINE(3).GT.0)  CALL LOAD(&56,F,I,M,X,REFINE(3),
                    LVESSL)
```

```
      IF(REFINE(4).GT.0)  CALL LOAD(&56,F,I,M,X,REFINE(4),
                    HBURET)
      IF(REFINE(5).GT.0)  CALL LOAD(&56,F,I,M,X,REFINE(5),
                    LBURET)
      IF(REFINE(6).GT.0)  CALL LOAD(&56,F,I,M,X,REFINE(6),
                    EZERO)
      IF(REFINE(7).GT.0)  CALL LOAD(&56,F,I,M,X,REFINE(7),
                    SLOPE)
C
C     CALCULATION OF THE SUM OF SQUARED RESIDUALS.
C
   20 I = 1
      J = NCONST
      ALFH = 0.000
   21 ALFH = ALFH + PK(J,1) * AL10
      BETA(I) = ALFH
      I = I + 1
      J = J - 1
      IF(J.GT.1)  GO TO 21
      BETA(I) = -PK(1,1) * AL10
C
C     IF(KOUNT.GE.4)  GO TO 60
C     WRITE(OUT,10008)  VZERO(1),HVESSL(1),LVESSL(1),
                    HBURET(1),
C    *  LBURET(1), EZERO(1), SLOPE(1)
C     WRITE(OUT,10008)  BETA
C
      THZERO = (HVESSL(1) + LVESSL(1) * NDPV) * VZERO(1)
      THBC = HBURET(1) + LBURET(1) * NDPB
      TLZERO = LVESSL(1) * VZERO(1)
      ALSLP = AL10 / SLOPE(1)
C
      F = 0.000
      DO 35 I=1,NP
      VOL = VZERO(1) + V(I)
      TT(1) = (THZERO + THBC * V(I)) / VOL
      TT(2) = (TLZERO + LBURET(1) * V(I)) / VOL
      ALFH = (E(I) - EZERO(1)) * ALSLP
      CX(1) = EXP(ALFH)
      ALFL = 1.0000
      DO 31 K=1,NBETAH
   31 ALFL = ALFL + EXP(BETA(K) + ALFH * K)
      CX(2) = TT(2) / ALFL
      ALFL = ALOG(CX(2))
      DO 32 K=1,NBETAH
   32 CI(K) = EXP(BETA(K)+ALFL+ALFH*K)
      CI(NCONST) = EXP(BETA(NCONST)-ALFH)
      THCALC = CX(1)
      DO 33 J=1,NCONST
   33 THCALC = THCALC + CI(J) * JOR(1,J)
      VCALC = (THZERO - THCALC * VZERO(1)) / (THCALC -
                    THBC)
      RESID = VCALC - V(I)
      IF(KOUNT.GT.0)  GO TO 35
C     WRITE(OUT,10008)  TT, CX, TOL, ALFL
```

```
C       WRITE(OUT,10008)  CI
        CALL ML(NCONST,2,TOL,2,BETA,CI,CX,TT,HX,TOLC,DT,DDT,
                  JQR)
        IF(IFAIL.GT.0)  GO TO 60
        ECALC = EZERO(1) + ALOG(CX(1)) / ALSLP
        ALFH = -ALOG10(CX(1))
        ALFL = ECALC - E(I)
        THCALC = E(I) + SLOPE(1) * ALFH
        SCALC = (EZERO(1) - E(I)) / ALFH
        WRITE(OUT,10001)   V(I), VCALC, E(I), ECALC, RESID,
                  ALFL, ALFH,
     1  CX(2), THCALC, SCALC
        IF(IFAIL.LT.JFAIL)  WRITE(OUT,10005)
        JFAIL = IFAIL
        TT(1) = (THZERO + THBC*(V(I)+0.004)) / VOL
        CALL ML(NCONST,2,TOL,2,BETA,CI,CX,TT,HX,TOLC,DT,DDT,
                  JQR)
        ALFH = ALFH + ALOG10(CX(1))
        ALFH = ABS(ALFH) * 60.0
        IF(ALFH.GT.0.3.AND.KOUNT.LE.0)  WRITE(OUT,10007)
   35 F = F + RESID ** 2
   50 IF(KOUNT.GT.0)  GO TO 55
        IF(IFAIL.NE.0)  GO TO 58
        RETURN
   55 WRITE(OUT,10008)  F, (X(I), I=1,M)
        IFAIL = 0
        JFAIL = 0
   56 RETURN
   58 WRITE(OUT,10006)
   60 WRITE(OUT,10002)
        IF(IFAIL.NE.0)  WRITE(OUT,10003)
        IF(IFAIL.LT.1)  WRITE(OUT,10004)
        IFAIL = 10
        JFAIL = 0
        RETURN 1
10001 FORMAT(' ',2F9.3,2F10.2,F12.3,F9.3,F13.3,1PE13.2,
                  0PF16.3,F11.3)
10002 FORMAT(' ','FAILURE IN SUBROUTINE CALC')
10003 FORMAT(' ','CAUSED BY NON-CONVERGENCE IN SUBROUTINE
                  ML')
10004 FORMAT(' ','THE MAXIMUM NUMBER OF ITERATIONS HAS
                  BEEN EXCEEDED.')
10005 FORMAT('+',118X,'?')
10006 FORMAT('0','A QUESTION MARK INDICATES A')
10007 FORMAT('+',120X,'UNBUFFERED')
10008 FORMAT(' ',1P8E12.3)
        END
```

```
        SUBROUTINE LOAD(*,F,I,M,X,IREF,ARRAY)
        DIMENSION  X(M), ARRAY(3)
        I = I + 1
        ARRAY(1) = X(I)
        IF(IREF.EQ.1)  RETURN
        IF(ARRAY(2).LT.X(I).AND.ARRAY(3).GT.X(I))  RETURN
        XA = (ARRAY(3) + ARRAY(2)) / 2.0
        XA = (X(I) - XA) / (ARRAY(3) - ARRAY(2))
```

```
      IF(XA.LT.0.000)  XA = - XA
      F = XA * 1.00E10
      RETURN 1
      END
-----------------------------------------------------------------
      SUBROUTINE ML(NK,NMBE,TOL,NC,HLNB,CI,CX,TT,HX,TOLC,
     1            DT,DDT,JQR)
      DIMENSION HLNB(NK),CI(NK)
      DIMENSION JQR(NMBE,NK)
      DIMENSION CX(NMBE),TT(NMBE),HX(NMBE)
      DIMENSION TOLC(NC),DT(NC),DDT(NC,NC)
      COMMON /FIVE/  JINP, JOUT, IFAIL, JFAIL, AL10, KOUNT
C          THIS ROUTINE CALCULATES ESTIMATES OF THE FREE
     1            CONCENTRATIONS O
C          LIGAND ETC.  USING A NUMBER OF MASS-BALANCE
     1            EQUATIONS EQUAL TO
C          NUMBER OF UNKNOWNS (THOSE FOR WHICH THERE IS NO
     1            POTENTIAL), TH
C          'NEWTON-RAPHSON' METHOD IS USED, WITH FIRST
     1            DERIVATIVES ONLY.
C          ESTIMATES ARE ALSO REQUIRED FOR THIS ROUTINE
     1            BUT 1.E-07 WILL
C          SUFFICE IF A MORE ACCURATE VALUE IS NOT
     1            AVAILABLE.
      NEMF = NMBE - NC
      IF(NEMF.EQ.0)  GO TO 103
      DO 102 I=1,NEMF
      IPNC = I + NC
  102 HX(IPNC)=ALOG(CX(IPNC))
  103 NCICL = 0
C          A CYCLE COUNTER, 100 CYCLES ARE PERMITTED AS
     1            MAXIMIMUM.
      DO 105 I=1,NC
C          TOLC(I) PROVIDES A RELATIVE TOLERANCE FOR USE
     1            WITH THE
C          CONVERGENCE CRITERION
  105 TOLC(I)=ABS(TT(I))*TOL
  121 NCICL=NCICL+1
      DO 125 J=1,NC
C          XC(J) IS ONE OF THE UNKNOWN CONCENTRATIONS THAT
     1            ARE BEING CALC
C          AS IT CANNOT TAKE A NEGATIVE VALUE, THE STEP
     1            LENGTH OF THE COR
C          VECTOR HX IS REDUCED SO THAT NONE OF THEM TAKES
     1            A NEGATIVE VAL
  122 IF(CX(J))123,123,125
  123 DO 124 I=1,NC
      HX(I)=0.5*HX(I)
  124 CX(I)=CX(I)-HX(I)
      GO TO 122
  125 CONTINUE
      DO 126 I=1,NC
      HX(I)=ALOG(CX(I))
C          DT(I) IS THE DIFFERENCE BETWEEN T OBSERVED AND
     1            T CALCULATED
```

```
C          FOR THE MASS-BALANCE EQUATION (I), I.E. IT IS
                   THE RESIDUAL.
  126 DT(I)=CX(I)-TT(I)
C
C     CHANGES AS RECOMMENDED BY LEGGETT  (TALANTA, 1978)
C
      DO 128 J=1,NK
      W=HLNB(J)
      DO 127 I=1,NMBE
  127 W=W+HX(I)*JQR(I,J)
C          CI(J) IS THE CONCENTRATION OF THE SPECIES (J)
                   DEFINED BY
C          THE INDICES IN JQR
      CI(J)=EXP(W)
      DO 128 I=1,NC
  128 DT(I)=DT(I)+JQR(I,J)*CI(J)
      IF(KOUNT.GT.1000)  RETURN
      DO 129 I=1,NC
C          CONVERGENCE CRITERION. WHEN ALL THE
                   MASS-BALANCE EQUATIONS
C          SATISFIED TO THE REQUIRED RELATIVE TOLERANCE,
                   CONTROL IS
C          PASSED BACK TO THE CALLING PROGRAM.
      IF(ABS(DT(I))-TOLC(I))129,129,131
  129 CONTINUE
      GO TO 190
  131 DO 152 I=1,NC
      DO 151 J=I,NC
C          DDT IS THE JACOBIAN FOR THE SYSTEM, AND IT IS
                   SYMMETRICAL AND
C          SQUARE.  ITS ELEMENTS ARE THE RELATIVE
                   DERIVATIVES, SO THAT
C          THEY ARE OBTAINED DIRECTLY FROM THE
                   CONCENTRATION TERMS
C          PREVIOUSLY CALCULATED.
      DDT(I,J)=0.
      DO 151 L=1,NK
      IF(JQR(I,L))149,151,149
  149 IF(JQR(J,L))150,151,150
  150 W=JQR(I,L)*JQR(J,L)*CI(L)
      DDT(I,J)=DDT(I,J)+W
  151 CONTINUE
  152 DDT(I,I)=DDT(I,I)+CX(I)
      CALL LINEQ(DDT,NC,DT,4)
      IF (IFAIL) 160,160,190
C          DT CONTAINS THE RELATIVE CORRECTIONS TO THE
                   PARAMETERS.
C                  HX WILL CONTAIN THE ABSOLUTE
                   CORRECTIONS.
  160 DO 165 I=1,NC
      HX(I)=-DT(I)*CX(I)
  165 CX(I)=CX(I)+HX(I)
C          IF 100 CYCLES HAVE BEEN EXCEEDED CONTROL IS
                   RETURNED TO CALC.
      IF(NCICL.LT.101)  GO TO 121
```

```
      IFAIL = IFAIL - 1
  190 RETURN
      END
      SUBROUTINE LINEQ(A,N,B,KFAIL)
      DIMENSION  A(N,N), B(N)
      COMMON /FIVE/  IN, OUT, IFAIL, JFAIL, AL10, KOUNT
C          SOLVES THE N SIMULTANEOUS LINEAR EQUATIONS
C                   A*X=B WITH M RIGHT-
C          SIDES IN B, THE SOLUTION VECTORS ARE LEFT IN B
C                   AND THE MATRIX
C          REPLACED BY ITS INVERSE, AFTER CHOLESKI
C                   FACTORING OF A TO GIVE
C          THE FORWARD SUBSTITUTIONS L*Y=B AND L*Z=E AND
C                   THE BACKWARD SUB
C          LT*X=Y AND LT*AINV=Z ARE PERFORMED
      IF (N-1) 455,5,9
    5 T=A(1,1)
      IF(T.LE.0)  GO TO 455
    6 A(1,1)=1./T
      B(1) = B(1) / T
      RETURN
    9 DO 80 I=1,N
      I1=I-1
      DO 70 J=I,N
      S=A(I,J)
      IF (I1) 10,30,10
   10 DO 20 K=1,I1
   20 S=S-A(I,K)*A(J,K)
   30 X=S
      IF (J-I) 60,40,60
   40 IF (X) 45,45,50
   45 IFAIL=KFAIL
      GO TO 400
   50 A(I,I)=1./SQRT(X)
      GO TO 70
   60 A(J,I)=X*A(I,I)
   70 CONTINUE
   80 CONTINUE
C          FORWARD SUBSTITUTION ON RIGHT HAND SIDES
      B(1) = B(1) * A(1,1)
      DO 120 I=2,N
      I1=I-1
      S = B(I)
      DO 110 K=1,I1
  110 S = S - A(I,K) * B(K)
  120 B(I) = S * A(I,I)
C          FORWARD SUBSTITUTION FOR INVERSION
      DO 170 J=1,N
      J1=J+1
      IF (J1-N) 140,140,170
  140 DO 160 I=J1,N
      I1=I-1
      S=0.
      DO 150 K=J,I1
  150 S=S-A(I,K)*A(J,K)
```

```
  160 A(J,I)=S*A(I,I)
  170 CONTINUE
C          BACKWARD SUBSTITUTION
      B(N) = B(N) * A(N,N)
      DO 220 J=1,N
  220 A(J,N)=A(J,N)*A(N,N)
      DO 290 II=2,N
      I=N-II+1
      T=A(I,I)
      I1=I+1
      S = B(I)
      DO 240 K=I1,N
  240 S = S - A(K,I) * B(K)
  245 B(I) = S * T
      DO 280 J=1,I
      S=A(J,I)
      DO 270 K=I1,N
  270 S=S-A(K,I)*A(J,K)
      A(J,I)=S*T
  280 CONTINUE
  290 CONTINUE
      DO 300 I=2,N
      I1=I-1
      DO 300 J=1,I1
  300 A(I,J)=A(J,I)
  400 RETURN
C
455   IFAIL=KFAIL
      RETURN
      END
```

```
C  ************************************************************
                    ************

C
C
C                              SUBPROGRAM CALIBT
C                              -----------------
C
C
C                          CALIBRATION AND ANALYSIS
C                            OF TITRATION DATA BY
C                          LINEAR BEST-FIT TECHNIQUES
C
C
C
C  ************************************************************
                    ************

C
C
C
C     THIS PROGRAM CALCULATES THE PH (AS THE NEGATIVE LOG
                  OF THE FREE
C     HYDROGEN CONCENTRATION) AT EACH POINT IN A
                  TITRATION AND THEN
C     DETERMINES THE GRADIENT AND INTERCEPT OF THE LEAST
                  SQUARES
```

```
C      BEST-FIT WITH RESPECT TO THE GLASS ELECTRODE
                  RESPONSE.
C      THE CALIBRATION IS IMPROVED BY OPTIMISATION OF THE
                  REAGENT
C      CONCENTRATIONS OR THE RELEVANT EQUILIBRIUM
                  CONSTANTS.
C      A GRAN PLOT ANALYSIS IS PROVIDED FOR COMPARISON.
C
C
C
C      THE FOLLOWING KINDS OF TITRATION, ALL INVOLVING
                  MONOBASIC
C      REACTANTS, ARE APPLICABLE.
C
C                  (1)   STRONG ACID VERSUS STRONG BASE.
C                  (2)   WEAK ACID VERSUS STRONG BASE.
C                  (3)   STRONG BASE VERSUS STRONG ACID.
C                  (4)   WEAK BASE VERSUS STRONG ACID.
C
C      STRONG ACID OR BASE MAY BE ADDED TO A CORRESPONDING
                  WEAK ACID
C      OR WEAK BASE IN THE VESSEL PRIOR TO TITRATION.
                  STANDARD ADDITIONS
C      ARE TREATED AS SUBSETS OF THE ABOVE TITRATION
                  SCHEMES.
C
C
C
C      THE NERNST SIGN CONVENTION IS TAKEN TO BE POSITIVE
C      (I.E. INCREASING PH GIVES DECREASING EMF VALUES.)
C      TITRATION VOLUMES MUST INCREASE MONOTONICALLY.
C
C
C
C      WITH STRONG ACID / STRONG BASE TITRATIONS, A SCAN
                  OF PKW CAN BE
C      IMPLEMENTED TO ESTIMATE AN OPTIMUM VALUE.
C      IT IS IMPORTANT TO OBTAIN AGREEMENT BETWEEN:
C      (1) THE OBSERVED AND THEORETICAL SLOPE FOR THE
                  ELECTRODE RESPONSE
C      AND (2) THE VALUES OF EZERO FOUND FOR DATA
                  CORRESPONDING TO THE
C      ACID RANGE COMPARED WITH THAT COVERING ALL BUFFERED
                  POINTS.
C      THESE ARE BETTER CRITERIA THAN A MINIMUM STANDARD
                  DEVIATION.
C      A SCAN OF THE LIGAND PROTONATION CONSTANT IS ALSO
                  POSSIBLE.
C
C
C**************************************************************
                  ************
C
C
C
```

```
C
      SUBROUTINE CALIBT(VZERO,EZERO,SLOPE,THZERO,TLZERO,
                  AHZERO,
     1  PKWMIN,PKWMAX,BLGMIN,BLGMAX)
C
C
C
      DIMENSION  W(100),H(100),PH(100),PHA(100),PHB(100),
                  G(100),VG(100),
     1  VOLT(100),X(100),Y(100),ET(100),EA(100),EB(100),
                  THT(100)
      INTEGER  IN, OUT, CARD
      REAL NERNST
      LOGICAL  LIGAND, ALKALI, ADJUST
C
C
C
      COMMON /TWO/ CI(7),CX(2),TT(2),HX(2),TOLC(2),DT(2),
                  DDT(2)
      COMMON /THREE/  V(100), E(100), N
      COMMON /FOUR/  BETA(7), ARRAY(3), TOL, NCONST,
                  NBETAH, JQR(2,7)
      COMMON /FIVE/  IN, OUT, IFAIL, JFAIL, AL10, KOUNT
      COMMON /SIX/  CARD(20), P(63)
C
      EQUIVALENCE (P(1),W(1))
C
C
C
  200 FORMAT('1')
  201 FORMAT('0')
  210 FORMAT(/'0',44X,'SUBPROGRAM CALIBT',/45X,17(1H-),
                  ////'0',20A4,//)
  211 FORMAT('0','THE INITIAL VOLUME IS',F7.2,/
     1' ','THE EXPECTED ELECTRODE INTERCEPT IS',F8.2,/
     2' ','THE EXPECTED NERNSTIAN SLOPE IS',F7.2)
  212 FORMAT('0','THE ACID CONCENTRATION IN THE TITRATION
                  VESSEL IS',
     11PE11.3,/' THE LIGAND CONCENTRATION IN THE
                  TITRATION VESSEL IS',
     21PE11.3,/' THE ACID CONCENTRATION IN THE BURETTE
                  IS',1PE11.3)
  213 FORMAT(' ','THE END POINT IS EXPECTED AT',F7.3)
  215 FORMAT(' ','THE VALUE OF PKW IS',F7.3,/'
                  ',26(1H-),/)
  216 FORMAT(' ','THE VALUE OF LOG BETAH IS',F7.3,/'
                  ',31(1H-),/)
  220 FORMAT('0','TITRATION POINT NUMBER',I3,' IS IN
                  ERROR',15X,2F12.2)
  222 FORMAT('0','INITIAL CONCENTRATION DATA PROBABLY
                  INCORRECT',//)
  231 FORMAT('0','THE GRAN-PLOT VALUES AT EACH POINT
                  ARE:',11X,'I',7X,
     1  'VOL',6X,'EMF',8X,'V(I)',12X,'G(I)',///)
  235 FORMAT('0','PKW IS IN ERROR')
```

```
  236 FORMAT('0','THE VALUE OF LOG BETAH IS IN ERROR')
  240 FORMAT('0','THE SEARCH FOR UNSUITABLE POINTS HAS
                 REMOVED ')
  241 FORMAT(' ',45X,I6,2F10.2,7(1PE15.4))
  245 FORMAT('+',45X,'NONE')
  250 FORMAT(' (50% OF THE POINTS, TAKEN ABOUT THE MIDDLE
                 OF THE SET)')
  251 FORMAT(' ','BEST-FIT INVOLVING',I3,' POINTS GIVES
                 EZERO =',
     1  F7.1,' (',F4.2,') AND A SLOPE =',F7.2,'
                 (',F4.2,')',/
     2 ' ','OVERALL STANDARD DEVIATION =',1PE11.3)
  252 FORMAT(///'0','USING DATA BEFORE THE ENDPOINT,')
  253 FORMAT('0','USING DATA AFTER THE ENDPOINT,')
  254 FORMAT('0','USING ALL THE BUFFERED DATA,')
  255 FORMAT(///'0',8X,'VOLUME'9X,'TH',12X,'PH',10X,
                 'E(OBS.)',
     1 7X,'E(CALC.)',8X,'RESIDUAL',5X,'PKW(CALC.)',4X,
                 'EZERO(CALC.)',//)
  256 FORMAT(' ',I3,F10.2,1PE15.3,0PF12.3,5F15.2)
  257 FORMAT('0','PKW(CALC.) IS OBTAINED USING THE
                 CURRENT VALUE FOR',
     1  ' EZERO (',F6.1,') AND THE NERNSTIAN SLOPE,
                 AVERAGE =',F7.3)
  258 FORMAT('0','EZERO(CALC.) IS OBTAINED USING THE
                 CURRENT VALUE FOR',
     1  ' PKW (',F6.2,') AND THE NERNSTIAN SLOPE,
                 AVERAGE =',F7.1,///)
  260 FORMAT('1',/'0',10X,35(1H*),5X,'NEW SCAN
                 ITERATION',5X,35(1H*),/)
  261 FORMAT('+','NOT REQUIRED.',/
     1  'THE RESULTS ARE INDEPENDENT OF THE VALUE OF
                 PKW.')
  262 FORMAT(//////'0',44X,'CALIBT CONCENTRATION
                 ADJUSTMENT')
  263 FORMAT(' ',43X,'AND TO DETERMINE A VALUE FOR PKW,')
  264 FORMAT(///'0','WITH A VESSEL-ACID CONCENTRATION
                 OF',1PE11.3,/' ',
     1  'AND A BURETTE-ACID CONCENTRATION OF',1PE11.3,/'
                 ',
     2  'THE FOLLOWING RESULTS ARE OBTAINED',////)
  265 FORMAT(' ','THE ENDPOINT NOW OCCURS AT',F7.3,///)
  266 FORMAT(' ',36X,'TO DETERMINE THE BURETTE-ACID
                 CONCENTRATION.',//)
  267 FORMAT(' ',37X,'TO DETERMINE THE VESSEL-ACID
                 CONCENTRATION')
C
C
C
   10 WRITE(OUT,200)
      LIGAND = .FALSE.
      ALKALI = .FALSE.
      ADJUST = .FALSE.
      NADJ = -10
C
```

```
      WRITE(OUT,210)  CARD
      NERNST = SLOPE
      WRITE(OUT,211)  VZERO, EZERO, NERNST
      IF(AHZERO.GT.0.000)  ALKALI = .TRUE.
      DUMMY = TLZERO
      IF(ALKALI)  DUMMY = -DUMMY
      ENDPT = -VZERO * (THZERO + DUMMY) / AHZERO
      ARESET = AHZERO
      TRESET = THZERO
      ERESET = ENDPT
      WRITE(OUT,212)  THZERO, TLZERO, AHZERO
      WRITE(OUT,213)  ENDPT
      IF(TLZERO.GT..1E-7)  LIGAND = .TRUE.
      PKW = PKWMIN
      WRITE(OUT,215)  PKW
      PKWINC = (PKWMAX - PKWMIN) / 5.000
      IF(PKWINC.LT.0.000)  PKWINC = 0.000
      BLGINC = 0.000
      IF(.NOT.LIGAND)  GO TO 16
      BLG = BLGMIN
      WRITE(OUT,216)  BLG
      IF(BLGMAX.GT.BLGMIN)  BLGINC = (BLGMAX - BLGMIN) /
     .          5.000
      IF(BLGINC.GT.0.000)  PKWINC = 0.000
   16 CONTINUE
C
C
C
      DO 20 I=2,N
      IF(V(I).LT.V(I-1))  GO TO 21
   20 CONTINUE
      GO TO 30
   21 WRITE(OUT,220) I, V(I), E(I)
      IF(I.EQ.2)  WRITE(OUT,222)
      GO TO 65
C
C
C
   30 DO 31 I=1,N
   31 H(I) = 10.000 ** ((E(I) - EZERO) / SLOPE)
      WRITE(OUT,231)
      IG = 1
      DO 32 I=1,N
      IF(LIGAND.AND.V(I).LT.0.00099)  GO TO 32
      IF(V(I).GT.ENDPT)  GO TO 33
      G(IG) = VZERO + V(I)
      IF(LIGAND)  G(IG) = V(I)
      HOLD = H(I)
      IF(ALKALI)  HOLD = 1.000 / HOLD
      G(IG) = G(IG) * HOLD
      VG(IG) = V(I)
      WRITE(OUT,241)  I, V(I), E(I), VG(IG), G(IG)
      IG = IG + 1
   32 CONTINUE
   33 IG = IG - 1
```

```
      INIT = 1
      IF(IG.GT.5)  CALL STRAIT(VG,G,W,IG,ENDPT,INIT,IFIN,
                  X,Y,OUT)
      NFLAG = IG + 1
      IF(NFLAG.GT.N-3)  GO TO 35
      IG = N - NFLAG + 2
      IF(NFLAG.GT.1)  WRITE(OUT,231)
      DO 34 I=NFLAG,N
      IG = IG - 1
      G(IG) = VZERO * V(I)
      HOLD = H(I)
      IF(.NOT.ALKALI)  HOLD = 1.000 / HOLD
      G(IG) = G(IG) * HOLD
      VG(IG) = V(I)
   34 WRITE(OUT,241)  I, V(I), E(I), VG(IG), G(IG)
      IG = N - NFLAG + 1
      INIT = N
      IF(IG.GT.5)  CALL STRAIT(VG,G,W,IG,ENDPT,INIT,IFIN,
                  X,Y,OUT)
C
   35 IF(PKW.GT.12.0.AND.PKW.LT.15.00)  GO TO 36
      IF(PKW.GT.0.5.OR.PKW.LT.-0.5)  WRITE(OUT,235)
      GO TO 65
   36 IF(.NOT.LIGAND)  GO TO 37
      IF(BLG.GT.2.00.AND.BLG.LT.12.00)  GO TO 40
      IF(BLG.GT.0.5.OR.BLG.LT.-0.5)  WRITE(OUT,236)
      GO TO 65
   37 TH2 = THZERO * 0.1
      IF(NADJ.GT.15)  TH2 = TH2 / 5.00
      TH1 = THZERO + TH2
      TH2 = THZERO - TH2
C
C
C
   40 IT = 0
      IA = 0
      IB = 0
      NFLAG = 0
      KWSCAN = 1
      THMOL = THZERO * VZERO
      IF(.NOT.(LIGAND.OR.ADJUST))  WRITE(OUT,215)  PKW
      IF(LIGAND)  WRITE(OUT,216)  BLG
      IF(.NOT.ADJUST)  WRITE(OUT,240)
      IF(.NOT.LIGAND)  GO TO 45
C
      TLMOL = TLZERO * VZERO
      IF(.NOT.ALKALI)  THMOL = THMOL + TLMOL
      DUMMY = -PKW
      WK = 10.00 ** DUMMY
      BETAH = 10.000 ** BLG
      DO 44 I=1,N
      W(I) = 1.000
      VOL = VZERO + V(I)
      TL = TLMOL / VOL
      TH = (THMOL + AHZERO * V(I)) / VOL
```

```
      HLO = -1.000
      HHI = -1.000
      HFREE = -1.000
      IF(TH.LE.0.000)  GO TO 41
C
C     ACID APPROXIMATION
C
      HOLD = 1.0 + BETAH * (TL - TH)
      HLO = HOLD ** 2 + 4.0 * BETAH * TH
      HLO = (SQRT(HLO) - HOLD) / (2.0 * BETAH)
      HFREE = HLO
   41 IF(TL.LE.TH)  GO TO 42
C
C     ALKALI APPROXIMATION
C
      HOLD = -(TH + WK * BETAH)
      HHI = HOLD ** 2 + 4.0 * BETAH * (TL - TH) * WK
      HHI = (SQRT(HHI) - HOLD) / (2.0 * BETAH * (TL - TH))
      IF(HFREE.LE.0.000)  HFREE = HHI
      IF(HLO.LT.1.00E-12.OR.HHI.LT.1.00E-12)  GO TO 42
C
      DUMMY = SQRT(WK)
      HFREE = -1.000
      IF(HLO.GT.DUMMY*100.0)  HFREE = HLO
      IF(HHI.LT.DUMMY/100.0)  HFREE = HHI
      IF(HFREE.GT.0.000)  GO TO 42
      HOLD = HLO * HHI / WK
      DUMMY = 1.000 / HOLD
      IF(HOLD.LT.1.000)  HOLD = 1.000
      IF(DUMMY.LT.1.000)  DUMMY = 1.000
      HLO = ALOG(HLO) * HOLD
      HHI = ALOG(HHI) * DUMMY
      HFREE = (HLO + HHI) / (HOLD + DUMMY)
      HFREE = EXP(HFREE)
   42 IF(HFREE.LT.1.0E-12)  GO TO 43
      FREEL = TL / (1.0 + BETAH * HFREE)
      FREELH = TL - FREEL
      IF((FREEL.LT.1.0E-3.OR.FREELH.LT.1.0E-3).AND.
     1  (HFREE.GT.1.0E-11.AND.HFREE.LT.1.0E-3))  GO TO 43
      IT = IT + 1
      PH(IT) = -ALOG10(HFREE)
      VOLT(IT) = V(I)
      THT(IT) = TH
      ET(IT) = E(I)
      IF(V(I).GT.ENDPT)  GO TO 421
      IA = IA + 1
      EA(IA) = E(I)
      PHA(IA) = PH(IT)
      GO TO 44
  421 IB = IB + 1
      EB(IB) = E(I)
      PHB(IB) = PH(IT)
      GO TO 44
   43 IT = IT + 1
      IF(IT.EQ.1)  WRITE(OUT,201)
```

```
      IT = IT - 1
      WRITE(OUT,241)  I, V(I), E(I)
   44 CONTINUE
      IF(IT.EQ.N)  WRITE(OUT,245)
      GO TO 52
C
   45 DO 48 I=1,N
      W(I) = 1.0000
      VOL = VZERO + V(I)
      TH = (THMOL + AHZERO * V(I)) / VOL
      IF(TH.LT.9.999E-4)  GO TO 46
      KWSCAN = 1
      IT = IT + 1
      IA = IA + 1
      ET(IT) = E(I)
      EA(IA) = E(I)
      PH(IT) = -ALOG10(TH)
      PHA(IA) = PH(IT)
      VOLT(IT) = V(I)
      THT(IT) = TH
      GO TO 48
   46 IF(TH.LE.-9.999E-4.AND.TH.GT.-0.01)  GO TO 47
      IF(ADJUST)  GO TO 48
      IT = IT + 1
      IF(IT.EQ.1)  WRITE(OUT,201)
      IT = IT - 1
      WRITE(OUT,241)  I, V(I), E(I)
      GO TO 48
   47 IT = IT + 1
      IB = IB + 1
      ET(IT) = E(I)
      EB(IB) = E(I)
      PH(IT) = PKW + ALOG10(-TH)
      PHB(IB) = PH(IT)
      VOLT(IT) = V(I)
      THT(IT) = TH
   48 CONTINUE
C
C
C
   50 IF(.NOT.ADJUST.AND.IT.EQ.N)  WRITE(OUT,245)
      IF(IT.LE.0)  STOP 50
      IF(IT.LE.5)  GO TO 54
      IF(IA.LT.5.OR.IB.LT.5)  GO TO 52
      CALL LINFIT(PHA,EA,W,IA,SLOPE,EZERO,SXSLP,SXINT,
                  STDDEV)
      SLOPE = -SLOPE
      ACIDSL = SLOPE
      IF(ADJUST)  GO TO 52
      IF(.NOT.ALKALI.OR.LIGAND)  WRITE(OUT,252)
      IF(.NOT.LIGAND.AND.ALKALI)  WRITE(OUT,253)
      IF(IB.EQ.0)  WRITE(OUT,250)
      WRITE(OUT,251)   IA, EZERO, SXINT, SLOPE, SXSLP,
                  STDDEV
C
```

```
      CALL LINFIT(PHB,EB,W,IB,SLOPE,EZERO,SXSLP,SXINT,
     1            STDDEV)
      SLOPE = -SLOPE
      IF(.NOT.ALKALI.OR.LIGAND)  WRITE(OUT,253)
      IF(.NOT.LIGAND.AND.ALKALI)  WRITE(OUT,252)
      IF(IA.EQ.0)  WRITE(OUT,250)
      WRITE(OUT,251)   IB, EZERO, SXINT, SLOPE, SXSLP,
     1            STDDEV
C
   52 IF(ADJUST.AND.NADJ.LT.10.AND.IA.GE.5.AND.IB.GE.5)
     1            GO TO 59
      CALL LINFIT(PH,ET,W,IT,SLOPE,EZERO,SXSLP,SXINT,
     1            STDDEV)
      SLOPE = -SLOPE
      IF(IA.LT.5.OR.IB.LT.5)  ACIDSL = SLOPE
      BASESL = SLOPE
      IF(ADJUST)  GO TO 59
      WRITE(OUT,254)
      WRITE(OUT,251)   IT, EZERO, SXINT, SLOPE, SXSLP,
     1            STDDEV
C
C
C
   54 WRITE(OUT,255)
      HOLD = 0.000
      DUMMY = 0.000
      PKWCLC = 0.000
      IF(IA.EQ.0)  IA = IT + 1
      DO 55 I=1,IT
      X(I) = PH(I)
      Y(I) = ET(I)
      ECALC = EZERO - SLOPE * PH(I)
      ERESID = ET(I) - ECALC
      EZOCLC = ET(I) + NERNST * PH(I)
      HOLD = HOLD + EZOCLC
      IF(I.LE.IA)  GO TO 55
      IF(I.EQ.IA+1)  WRITE(OUT,201)
      PKWCLC = ((EZERO - ET(I)) / NERNST) - PH(I) + PKW
      DUMMY = DUMMY + (1.0 / 10.00 ** PKWCLC)
   55 WRITE(OUT,256)  I, VOLT(I), THT(I), PH(I), ET(I),
     1            ECALC, ERESID,
     1    PKWCLC, EZOCLC
C
      WRITE(OUT,201)
      IF(IT.LE.5.OR.IA.LT.5)  GO TO 65
      IF(IB.LT.3)  GO TO 56
      PKWCLC = DUMMY / FLOAT(IB)
      PKWCLC = -ALOG10(PKWCLC)
      WRITE(OUT,257)  EZERO, PKWCLC
   56 EZOCLC = HOLD / FLOAT(IT)
      WRITE(OUT,258)  PKW, EZOCLC
C
C
C
   59 IF(LIGAND)  GO TO 60
```

```
      NADJ = NADJ + 1
      IF(NADJ-11)  591, 592, 594
  591 ADJUST = .TRUE.
      ACIDSL = ACIDSL - NERNST
      IF(NERNST.LT.0.00)  ACIDSL = -ACIDSL
      IF(ACIDSL.GT.0.000)  TH1 = THZERO
      IF(ACIDSL.LT.0.000)  TH2 = THZERO
      THZERO = (TH1 + TH2) / 2.000
      GO TO 40
  592 IF(.NOT.ADJUST)  GO TO 593
      ENDPT = -VZERO * THZERO / AHZERO
      WRITE(OUT,262)
      WRITE(OUT,267)
      IF(IB.GT.3)  WRITE(OUT,263)
      WRITE(OUT,201)
      WRITE(OUT,264)  THZERO, AHZERO
      WRITE(OUT,265)  ENDPT
      ADJUST = .FALSE.
      NADJ = 10
      GO TO 40
  593 ADJUST = .TRUE.
      TH2 = AHZERO * 0.1
      IF(NADJ.GT.15)  TH2 = TH2 / 5.00
      TH1 = AHZERO + TH2
      TH2 = AHZERO - TH2
      NADJ = NADJ + 1
  594 IF(NADJ-21)  597, 37, 595
  595 IF(NADJ-31)  591, 593, 596
  596 IF(NADJ-40)  597, 598, 60
  597 BASESL = BASESL - NERNST
      IF(NERNST.LT.0.00)  BASESL = -BASESL
      IF(BASESL.GT.0.000)  TH2 = AHZERO
      IF(BASESL.LT.0.000)  TH1 = AHZERO
      AHZERO = (TH1 + TH2) / 2.000
      THZERO = -AHZERO * ENDPT / VZERO
      GO TO 40
  598 IF(IA.LT.5.OR.IB.LT.5)  GO TO 65
      WRITE(OUT,262)
      WRITE(OUT,266)
      WRITE(OUT,264)  THZERO, AHZERO
      WRITE(OUT,265)  ENDPT
      ADJUST = .FALSE.
      GO TO 40
C
C
C
   60 IF(PKWINC.LE.0.0001)  GO TO 62
      NADJ = 0
      PKW = PKW + PKWINC
      IF(PKW.GT.PKWMAX)  GO TO 62
      WRITE(OUT,260)
      IF(KWSCAN.EQ.0)  WRITE(OUT,260)
      IF(KWSCAN.EQ.0)  GO TO 62
      THZERO = TRESET
      AHZERO = ARESET
```

```
      ENDPT = ERESET
      WRITE(OUT,264)  THZERO, AHZERO
      WRITE(OUT,213)  ENDPT
      GO TO 35
C
   62 IF(.NOT.LIGAND.OR.BLGINC.LE.0.0001)  GO TO 65
      BLG = BLG + BLGINC
      IF(BLG.GT.BLGMAX)  GO TO 65
      WRITE(OUT,260)
      GO TO 35
C
   65 WRITE(OUT,200)
      RETURN
      END
```

```
C
C
C
C
C
C     THE FOLLOWING SUBROUTINE SEARCHES DATA STORED IN
                THE ARRAYS
C     NAMED V AND G TO FIND THE SEGMENT GIVING THE
                STRAIGHTEST LINE.
C     ONLY THOSE LINES WITH ABSSICA INTERCEPTS WITHIN
                PTOL PERCENT OF
C     THE ESTIMATED END POINT VALUE ARE ACCEPTED;
                IF THIS CONDITION IS
C     SATISFIED, THE SEGMENT GIVING THE LOWEST STANDARD
                DEVIATION IS
C     LOCATED;  OTHERWISE, THE LINE FOUND IS THAT GIVING
                THE CLOSEST
C     AGREEMENT WITH THE END POINT ESTIMATE.
                SUBROUTINE LINFIT IS
C     CALLED BETWEEN 20 AND 50 TIMES;  ITER DETERMINES
                HOW FINELY
C     THE DATA IS DIVIDED INTO LINE SEGMENTS.
C
C
C
C
C
      SUBROUTINE STRAIT(V,G,W,N,ENDPT,INIT,IFIN,X,Y,OUT)
      DIMENSION  V(N), G(N), W(N), X(N), Y(N)
      INTEGER OUT
C
C
C
  201 FORMAT(///'0','GRAN-PLOT EXTRAPOLATIONS',/'
                ',24(1H*),///'0',
     1  2X,'SEGMENT',8X,'END PT.',5X,'STD. DEV.',//)
  202 FORMAT(' ',I3,2X,'-',I4,4X,F10.4,3(1PE15.4))
  203 FORMAT(//'0','THE STRAIGHTEST SEGMENT
                GIVES',3X,F10.4,/'0',
     1 'THE MINIMUM STD. DEVIATION GIVES',3X,F10.4)
```

```
  205 FORMAT('+',48X,'OMITTED BECAUSE THE ESTIMATED
                  ENDPOINT IS OUTSIDE
     1THE PERMITTED RANGE OF', F5.1,'%')
  207 FORMAT('0','THE WEIGHTED AVERAGE GIVES',3X,F8.4,
                  /'0',
     1  'THE ACTUAL AVERAGE GIVES',3X,F8.4,////)
  210 FORMAT(' FAILED TO FIND THE ENDPOINT',//)
  211 FORMAT(' CLOSEST AGREEMENT WITH THE ESTIMATED VALUE
                  IS WITHIN',
     1  F6.1,'%',//)
  215 FORMAT('0ANOTHER ATTEMPT WITH INCREASED ENDPOINT
                  RANGE',///)
  218 FORMAT('0',I5,' POINTS HAVE BEEN OMITTED BECAUSE
                  THEIR ESTIMATED
     1ENDPOINTS ARE OUTSIDE A RANGE OF',F6.1,'%',///)
C
C
C
      PTOL = 5.0
      ISTART = INIT
    5 ITER = 5
      IF(ISTART.EQ.1)  ISTART = -1
      INIT = 0
      ENDOLD = 1.000E19
      STDOLD = 1.000E19
      STDSTD = 1.000E19
      SAVINT = 0.000
      SAVSLP = 0.000
      KOUNT = 0
      WGTAVE = 0.000
      ENDAVE = 0.000
      STDAVE = 0.000
      NDUD = 0
C
      IF(N.LT.6)  STOP
      MMIN = 5
      IF(N.GT.12)  MMIN = 6
      IF(N.GT.25)  MMIN = 7
      IF(N.GT.50)  MMIN = 9
      NM1 = N - 1
      MINC = (N - MMIN) / ITER
      IF(MINC.LT.1)  MINC = 1
      DO 10 I=1,N
   10 W(I) = 1.000
      IF(PTOL.LT.7.00)  WRITE(OUT,201)
      DO 35 M=MMIN,NM1,MINC
      MP1 = M + 1
      IINC = ((N - M) / ITER)
      IF(IINC.LT.1)  IINC = 1
C
      DO 35 I=M,N,IINC
      DO 20 J=1,M
      K = I + J - M
      X(J) = V(K)
   20 Y(J) = G(K)
```

```
      J = I - M + 1
      CALL LINFIT(X,Y,W,M,SLOPE,XINT,SXSLP,SXINT,STDDEV)
      ENDTRY = -XINT / SLOPE
      ENDAVE = ENDAVE + ENDTRY
      WGTAVE = WGTAVE + ENDTRY / STDDEV
      STDAVE = STDAVE + 1.000 / STDDEV
      KOUNT = KOUNT + 1
      K = IABS(ISTART-J+1)
      L = IABS(ISTART-I+1)
      IF(PTOL.LT.7.00)  WRITE(OUT,202)  K, L, ENDTRY,
                        STDDEV
      ENDNEW = (ABS(ENDTRY-ENDPT) / ENDPT) * 100.0
      IF(ENDNEW.LT.PTOL)  GO TO 30
      IF(PTOL.LT.7.00)  WRITE(OUT,205)  PTOL
      ENDAVE = ENDAVE - ENDTRY
      KOUNT = KOUNT - 1
      WGTAVE = WGTAVE - ENDTRY / STDDEV
      STDAVE = STDAVE - 1.000 / STDDEV
      NDUD = NDUD + 1
      IF(ENDNEW.GT.ENDOLD)  GO TO 35
      ENDOLD = ENDNEW
      GO TO 32
   30 ENDOLD = 0.0000
      IF(STDDEV/FLOAT(I-J+2).GT.STDOLD)  GO TO 34
   32 INIT = J
      IFIN = I
      SAVINT = XINT
      SAVSLP = SLOPE
      STDOLD = STDDEV / FLOAT(I-J+2)
   34 IF(STDDEV.GT.STDSTD)  GO TO 35
      STDSTD = STDDEV
      SAVEND = ENDTRY
   35 CONTINUE
      IF(KOUNT.GE.1)  GO TO 38
      WRITE(OUT,210)
      IF(ENDOLD.LT.1.0E2)  WRITE(OUT,211)  ENDOLD
   36 IF(PTOL.GT.7.0)   GO TO 39
      PTOL = PTOL * 2
      IF(ENDOLD.GT.PTOL) RETURN
      WRITE(OUT,215)
      GO TO 5
   38 STDDEV = STDOLD * FLOAT(IFIN-INIT+2)
      ENDTRY = -SAVINT / SAVSLP
      ENDAVE = ENDAVE / FLOAT(KOUNT)
      WGTAVE = WGTAVE / STDAVE
      WRITE(OUT,203)  ENDTRY, SAVEND
      WRITE(OUT,207)  WGTAVE,  ENDAVE
      IF(FLOAT(NDUD).GT.FLOAT(KOUNT)/2.0)  GO TO 36
      RETURN
   39 WRITE(OUT,218)  NDUD, PTOL
      RETURN
      END
C
C
C  LINEAR LEAST SQUARES FIT ROUTINE
```

```
C  ---------------------------------
C
C
C
C  DEFINITION OF VARIABLE NAMES:
C
C
C       X, Y                = DATA ARRAYS
C       W                   = ARRAY FOR WEIGHTING FACTORS
C       N                   = NUMBER OF DATA POINTS
C       NW                  = FLAG FOR WEIGHTING
C       XINT, XSLOPE        = INTERCEPT AND SLOPE OF LEAST
                    SQUARES LINE
C       SXINT, SXSLP        = STANDARD DEVIATIONS OF XINT AND
                    XSLOPE
C       STDDEV              = STANDARD DEVIATION OF THE LINE
                    FIT
C
C
C
      SUBROUTINE LINFIT(X,Y,W,N,XSLOPE,XINT,SXSLP,SXINT,
                    STDDEV)
      DIMENSION X(N), Y(N), W(N)
      WW = 0.000
      WX = 0.000
      WY = 0.000
      WXY = 0.000
      WXX = 0.000
      WYY = 0.000
      DO 10 I=1,N
      WW = WW + W(I)
      WY = WY + W(I) * Y(I)
      WX = WX + W(I) * X(I)
      WXY = WXY + W(I) * X(I) * Y(I)
      WXX = WXX + W(I) * X(I) ** 2
   10 WYY = WYY + W(I) * Y(I) ** 2
      DENOM = WW * WXX - WX ** 2
      XSLOPE = (WW * WXY - WX * WY) / DENOM
      XINT = (WXX * WY - WX * WXY) / DENOM
      VSUM = 0.000
      DO  20  I=1,N
   20 VSUM = VSUM + (Y(I) - XINT - XSLOPE * X(I)) ** 2
      SS = VSUM / FLOAT(N-2)
      STDDEV = SQRT(SS)
      SXINT = SQRT((SS/WW)*(1.0+((WX**2)/DENOM)))
      SXSLP = SQRT(SS*WW/DENOM)
      RETURN
      END
```

APPENDIX A5

Instructions and FORTRAN listing of the computer program FORMAT

FORMAT

DATA PREPARATION FOR MINIQUAD, SCOGS, ZPLOT, PSEUDOPLOT, COMICS, ECCLES AND MAGEC COMPUTER PROGRAMS :-

A COMMON INPUT PROCEDURE

This document describes how the input data for programs in the Bio-inorganic Chemistry computer library held by the South West Universities Computer Network should be punched onto data cards. It refers only to those equilibrium systems in which there are not more than one kind of metal ion and two kinds of ligand. Glass electrode potentiometric data can be analysed using the MINIQUAD, SCOGS, ZPLOT, PSEUDOPLOT or MAGEC computer programs. Complex distributions as a function of pH can be calculated using the COMICS or ECCLES programs. ZPLOT, PSEUDOPLOT and COMICS can produce graphical output. Separate documents giving details on each of the above programs, one describing the Job Control commands required to call and execute the programs, and one about graphical procedures are available.

INDEX

Section 1: Introduction

A variety of computer programs are generally used to process data from or pertaining to potentiometric titrations of bioinorganic equilibrium systems. While the same kind of data is needed in each case, the programs have different requirements concerning the way in which their input data must be prepared. To avoid having to punch a separate deck of cards for each program that one wants to use, a program called FORMAT may be executed first so that data prepared in a standard way is transformed into the particular layout required by the user.

The program for which data is to be prepared is referred to hereunder as the main program. The data generated for it by program FORMAT is stored in a temporary computer file and is not normally seen by the user. The printed output from program FORMAT is usually produced only if an error has been detected and the execution of the main program has consequently been abandoned.

Program FORMAT can deal with equilibrium systems having the following components.

(a) proton only
(b) ligand and proton
(c) ligand, metal ion and proton
(d) two ligands, metal ion and proton

Up to 20 complexes may be specified by the user.

Potentiometric data can be accepted if the titrand and titrant are solutions of any one or a combination of the following components.

(a) a strong acid or a strong base
(b) a ligand
(c) a second ligand
(d) a metal ion

A maximum of 100 data points per titration and an overall total of 500 points can be accommodated.

Most programs in the Bio-inorganic computer library do not permit such a wide range of input possibilities. It is the responsibility of the user to ensure that the main program which is specified will be compatible with the system as defined for program FORMAT. This also applies to options made available by program FORMAT. Incompatible or inapplicable data may be ignored or they may cause execution of the main program to be terminated.

Section 2: General input requirements

The input for program FORMAT is intended to be general, easy to prepare and above all systematic. Broadly, the program requirements may be categorised as follows (the section numbers used here are cross-referenced throughout this document and in the print out produced by the program).

1. The name of the main program for which data is to be prepared (see Section 3).

2. A title for identification purposes (see Section 4).

3. Definition of the equilibrium system in terms of the complex formation constants or estimates thereof when this is applicable (see Section 5).

4. Definition of the equilibrium system in terms of the concentrations of components or estimates thereof when this is applicable (see Section 6).

5. The glass electrode parameters for each titration or estimates thereof when this is applicable (see Section 7).

6. The titration data, usually in terms of millilitres and millivolts (see Section 8).

7. An input terminator (see Section 9).

The above order is mandatory but it may be appropriate to omit category 6. When a series of titrations is involved, categories 4, 5 and 6 are repeated as often as required.

All cards except those bearing the actual titration data have the same basic format: columns 1 through 5 contain a keyword - a string of characters and/or digits - which serves to identify the data appearing in subsequent fields on that card. Recognisable keywords for each category required by the program are given in the appropriate sections below.

With one exception, detailed in Section 4, the data on all cards containing a keyword is entered as one or two digits in columns 7 and 9 respectively and one or more "real" numbers in columns 11 through 20, 21 through 30, 31 through 40, etc, In computer terminology, a "real" number is one which
(a) has a decimal point and
(b) may have an exponent - if so, this is signified by an 'E' placed directly after the number containing the decimal and immediately before the appropriate power of ten by which the number must be multiplied. If the real number has an exponent, it must be "right justified", (i.e. positioned as far as possible towards the righthand side of the field so that the last digit of the power of ten appears in the last column

of the field.) If there is no exponent, the real number may be positioned anywhere inside each field. A value of zero is assumed if the number is omitted.

The general FORTRAN statement for input format may thus be represented by:
(5A1, 1X, I1, 1X, I1, 1X, 7G10.2)

Some examples are given below.

```
KEYWD  1 4 8.1759E-13   10.000   8.652

KEYWD  1     22.00   2.22222E12

KEYWD       1.000        1.0E7

KEYWD

KEYWD        0.00463
```

Section 3: Program keywords

The first card read by program FORMAT specifies the main program for which data is to be prepared.

The following keywords are recognised.

Keyword (cols 1-5)	Meaning
MINIQ MINI1 MINI2	Call main program MINIQUAD. MINIQ and MINI1 refer to the original version of this program while MINI2 calls MINIQUAD (75)
SCOGS	Call main program SCOGS
COMIC	Call main program COMICS
PPLOT	Call main program PSEUDOPLOT
ZPLOT	Call main program ZPLOT
MAGEC	Call main program MAGEC
ECCLS	Call main program ECCLES
CHECK.	No main program required (FORMAT will just check the data)

Only one such keyword may appear. In other words, it is not possible to prepare output for more than one main program on each occasion FORMAT is executed.

The general format for the rest of the card is as follows.

Integer field (cols 7 + 9)	-	Digit or digits indicating which of the options provided by the main program are required. (Called IPROG and JPROG respectively)
Real field (cols 11-20, 21-30 etc)	-	Data required by the main program options (e.g. as specified in the integer field). Usually graph plotting parameters.

Specific details appropriate to each main program can be found in Sections 11 through 19.

Section 4: Title keyword

Most of the main programs require a title or some other data for identification purposes.

The data field following the keyword 'TITLE' is given a special format so that any alphanumeric string is acceptable. The data should be placed in columns 11 through 76.

In most cases, the alphanumeric string is transferred directly to the main program which simply prints it out as a heading. A title must be supplied, however, even when the main program does not require one.

N.B. The last four columns on this title card are reserved for user identification. See PMM or DRW about this. If program FORMAT is called by the BIONIC Macro, this identification is automatically transferred from the macro to this card image. Thus, users need not actually punch the information in these columns.

EXCEPTIONAL FORMAT: (5A1, 5X, 70A1)

Section 5: Formation constants

The formation constant data is headed by a card on which one of the keywords 'LBETA' or 'BETAF' must be punched. (The usual number fields on these cards are ignored.) The leader keyword is followed by a series of cards on each of which appears a string of digits defining the composition of a complex and a value for the appropriate formation constant. The keyword 'LBETA' indicates that log values are supplied while 'BETAF' signifies that the formation constants themselves are provided.

The formation constant or its log value is placed in the first "real" number field (columns 11 through 20).

The string of digits representing the composition of each complex species comprises the keyword and occupies up to 5 columns starting in column 1. The number of times each component appears in each complex (i.e. its multiplicity) must be given as follows:

(i) the multiplicity of the ligand or ligands;
(ii) the multiplicity of the metal ion, if there is one
(iii) the multiplicity of protons in the complex.

Each figure must be a single digit, although that referring to the number of protons may be signed or unsigned. The string should contain no spaces. It will thus occupy less than 5 columns when the metal ion and/or second ligand are absent or when the last digit is unsigned. However, every component present in the system must be represented in the digit string defining each complex species, i.e. a zero is used whenever the component is not part of the complex being defined. The number of protons in the complexes must always be specified, even in those (unusual) computations where protonation constants are not involved. The multiplicity of the ligand must also always be represented.

In general, a card image having the form

abcd logbeta

gives rise to a complex species concentration calculated as follows.

complex concentration=beta.(ligand 1)^{a}.(ligand 2)^{b}.(metal)^{c}.(h)^{d}

When the metal ion or second ligand are absent, the corresponding terms in the above expressions disappear.

Section 6: Concentration data

A distinction is made at this stage between those main programs required to calculate equilibrium distributions and those which are to process titration data. COMICS and ECCLES can only determine equilibrium concentrations; PSEUDOPLOT can do both; all the other main programs require titration data.

A. Equilibrium simulations

The concentration data is headed by a card on which the keyword 'CONCS' must be punched. (The usual number fields following the keyword on this card are ignored.)

The following keywords referring to concentration data for equilibrium simulations are recognised.

Keyword (cols 1-5 on cards following 'CONCS')	Meaning
ACIDT	Total concentration of strong acid in the equilibrium system. A negative quantity is meaningful; referring to an excess of hydroxide ion or hydroxo-complexes.
LIG1T	Total concentration of ligand in the equilibrium system.
LIG2T	Total concentration of the second ligand in the equilibrium system, if applicable.
METLT	Total concentration of metal ion in the equilibrium system, if applicable.
ACIDF	Free hydrogen ion concentration in the equilibrium system.
LIG1F	Free concentration of ligand in the equilibrium system.
LIG2F	Free concentration of the second ligand in the equilibrium system, if applicable.
METLF	Free concentration of the metal ion in the equilibrium system, if applicable.
SCNPH SCNPA	See note (3) below.

The order of the keywords following 'CONCS' is not important. Omit those which are not applicable (e.g. when the concentration is zero).

Note that if the multiplicity of the protons is negative, an hydroxo-complex is being specified: the calculation of the complex concentration involves taking a reciprocal of the free hydrogen ion concentration. The concentration of free hydroxide ion is thus calculated from the dissociation constant of water (say, $pK_w = 14.00$) as follows.

```
0-1          -14.00
```

Some other examples are

```
00-1         -14.00  }
101            9.20  }   three component system:
102           11.68  }   ligand, metal and proton
110            6.04  }
111           10.11  }
210           10.60  }
11-1           0.50  }

11             5.80  }   two component system:
12             8.27  }   ligand and proton only
0-1          -14.00  }
```

In respect of those programs which calculate or optimise formation constant values, the digit '1' placed in column 7 flags the complex species in question for refinement of its formation constant.

A digit '2' placed in column 7 also indicates the formation constant is to be refined but in this case the value will only be permitted to vary between the limits provided in columns 21-30 (lower value) and 31-40 (upper value). This option is valid only for program MAGEC. Otherwise, it has the same effect as the digit '1'.

The order of the formation constants is unimportant.

The art in determining or using formation constants lies in choosing the correct model. Great care must be exercised to ensure that <u>all</u> significant complex species (including metal-hydroxy complexes, protonated ligand species as well as the various metal-ligand-proton complexes) appear in the set prepared for simulation or refinement programs. Not all formation constants should necessarily be optimized simultaneously. Beginners will require help with these matters.

The order of the keywords following 'CONCS' is not important. The general numerical format is as follows.

Integer field (cols 7 + 9) - Digit in column 7 indicating whether the concentration of a component is to be scanned. (See below for further details.) For each ligand concentration, the value of NDP is required in column 9 (see note (4) below).

Real fields - The concentrations of the components in question in columns 11 through 20.

If the concentration is to be scanned, the initial value is increased till its exceeds a maximum value provided in columns 21 through 30. The digit '3' in column 7 indicates that the concentration is to be incremented additively by the amount specified in columns 31 through 40. The digit '4' in column 7 indicates that the concentration is to be multiplied by the factor in columns 31 through 40.

Notes: (1) Only one component may be flagged for a concentration scan.

(2) Either the free concentration or the total concentration of each component should be provided, not both.

(3) The main programs COMICS and PSEUDOPLOT permit the calculation of equilibrium concentrations at a number of pH values specified by the user. To exercise this option, the keyword 'SCNPH' replaces 'ACIDF'.
The pH value is provided in columns 11 through 21. More than one 'SCNPH' data point may be provided in this way.

PSEUDOPLOT offers a similar facility for a scan of pA (negative log of the free ligand concentration). In this case, the keyword is 'SCNPA'. This replaces 'LIG1F'. Implementation of either the 'SCNPH' or the 'SCNPA' options precludes scanning the concentration of any other component.

(4) The number of dissociable protons (NDP) for each ligand (i.e. parent acid) in the form they were added to the solution.

For example,	NDP
(a) histamine	0
(b) glycine	1
(c) sodium glycinate	0
(d) succinic acid	2
(e) phosphoric acid	3
(f) sodium dihydrogen phosphate	2
(g) disodium hydrogen phosphate	1
(h) trisodium phosphate	0

B. Titration data processing

For titrations, the concentrations of components in the system are defined as follows.

(1) The concentrations of components in the titration vessel. This data is headed by a card on which the keyword 'VESSL' must be punched.

(2) The concentrations of components in the burette. This data is headed by a card on which the keyword 'BURET' must be punched.

The above order is important - titration vessel data must be entered before that referring to the concentrations in the burette. (The usual number fields (following the keywords 'VESSL' and 'BURET' are ignored.)

The following keywords are recognised.

Keyword (on cards following 'BURET')	Meaning
INITV	Initial volume in the titration vessel
ACIDV	Initial concentration of strong acid in the titration vessel. A negative quantity refers to strong alkali.
LIG1V	Initial concentration of ligand in the titration vessel, if applicable.
LIG2V	Initial concentration of the second ligand in the titration vessel, if applicable.
METLV	Initial concentration of metal ion in the titration vessel, if applicable.
ACIDB	Concentration of strong acid in the burette. A negative quantity refers to strong alkali
LIG1B	Concentration of ligand in the titration vessel, if applicable.
LIG2B	Concentration of the second ligand in the burette, if applicable.
METLB	Concentration of the metal ion in the burette, if applicable.

The order of the keywords following 'VESSL' or 'BURET' is not important. Omit those which are not applicable (e.g. when the concentration is zero).

The order of the keywords following 'VESSL' and 'BURET' is not important. The general numerical format is as follows.

Integer field (cols 7 + 9) - Either of the digits '1' or '2' in column 7 causes refinement of that concentration by program MAGEC, in the same way as described for the refinement of formation constants in Section 5.

For each ligand concentration, the value of NDP is provided in column 9 (see note (4) above). Otherwise, this column is ignored.

Real field - The value or best estimate for the concentration in columns 11 through 20. If required for MAGEC refinement, a lower limit in cols 21-30 and an upper limit in cols 31-40.

A value of zero is assumed if the figure is omitted.

Section 7: Glass electrode parameters

The glass electrode parameters are headed by a card on which the keyword 'GLASS' must be punched. (The usual numerical fields on this card are ignored.) It is followed by data which defines or estimates the intercept and slope of the Nernstian equation for the glass electrode response to free hydrogen ion concentration.

$$E = E_o \pm \text{slope.} \log[H^+]$$

The following keywords are recognised.

Keyword (in cols 1-5 on cards following 'GLASS')	Meaning
EZERO	The standard emf of the glass electrode (i.e. the emf corresponding to $\log[H^+] = 0$.
SLOPE	The gradient of the Nernstian equation having $\log[H^+]$ as the independent variable.
TEMPC	The temperature of the solution in celcius. Optional. This value may be supplied instead of the slope in which case a theoretical gradient is calculated. Otherwise a theoretical value is calculated only for purposes of comparison.

If 'TEMPC' is given instead of the slope it must be preceded by 'EZERO'. The signs of E_o and slope must be the same.

The general numerical format is as follows.

Integer field (cols 7-9) - The digits '1' or '2' in column 7 cause refinement of either EZERO or SLOPE by program MAGEC in the same way as described in Section 5.

Real field - The value or best estimate for the glass electrode parameter in columns 11 through 20. If required for MAGEC refinement, a lower limit in cols 21-30 and an upper limit in cols 31-40.

Section 8: Titration data

Each set of titration data must be headed by a card on which one of the keywords 'ML/MV' or 'ML/PH' is punched. (The usual numerical fields on these cards are ignored.) The header is followed by a series of cards, one for each titration point, showing two "real numbers" without exponents and separated by a comma.

The keyword 'ML/MV' indicates that the data which follows are pairs of millilitre and millivolt readings (in that order). The keyword 'ML/PH' signifies that the pairs are millilitre and pH values.

The data are unformatted apart from the delimiting comma. That is, the numbers may be positioned anywhere in columns 1-20 with or without spacing. It is however advisable to keep the data in columns 1-10 whenever possible because this improves error diagnosis by the program.

A set of titration data is terminated by -

(a) the keyword 'FINIS' (see Section 9)

(b) the definition of a new titration in terms of different component concentrations (see Section 6). This is indicated by the keyword 'VESSL'

(c) the physical end of the data.

A maximum of 100 data points per titration may be entered. As none of the main programs can accept more than 500 data points in total, program FORMAT also tests to determine whether this limit has been exceeded.

Titre volumes must increase monotonically. Changes in emf must always be in the same sense. Program FORMAT will register a sequence error if either of these conditions is violated. Mispunched data is the usual cause of this.

Section 9: Termination of input

Input to program FORMAT is properly terminated by a card on which the keyword 'FINIS' has been punched.

A digit in the first integer field (col.7) indicates that the main program should not be executed and that the intermediate data file should be printed.

Section 10: Error diagnostic procedures

Program FORMAT will usually continue processing input data after errors have been detected. Thus, remaining data is at least partially checked although execution of the main program is to be aborted.

When the program detects an error it usually ignores the card in question and proceeds to input the next one. If this generates a second error or the program finds itself out of sequence, the section number becomes ill-defined and all further data cards are rejected until a recognisable keyword is encountered. Obviously, no checking procedures can be applied to data which is ignored in this way.

The failure of program FORMAT to recover from an error in the data will in all probability have been caused by a transposition of the input card images.

It has previously been stated that the user must ensure compatibility between the system as defined for program FORMAT and that which can be accepted by the specified main program. Although certain checks are made, the consequences of this kind of error are unpredictable. If, however, such an incompatibility is detected one of the following codes will be generated by program FORMAT and execution of the main program will be terminated.

Mode code	Error
1	Burette concentrations are required but are not provided.
2	The concentrations refer to titration data and not to equilibrium simulations
3	The concentrations refer to equilibrium simulation and not to the processing of titration data
10	The number of system components is incompatible with the specified main program
20	The attempt to refine a parameter is not permitted by the specified main program
25	pK_w cannot be refined by the specified main program
30	The number of titration data sets is incompatible with the specified main program
40	Two kinds of ligand are not permitted by the specified main program
45	More than 1 component is required by the specified main program

48	A value for pK_w is required by the specified main program. No value is provided or the value is unreasonable (e.g. check the sign)
50	At least one formation constant is required to be (nominally) refined by the specified main program
55	A formation constant value required by the specified main program has been omitted
70	Dimension limits of the main program would be exceeded
81	The scan mode required by the user is not permitted by the specified main program
90	Total concentrations are required by the specified main program but free concentrations have been provided
95	The main program does not permit changes in NDP between one titration and another
96	The main program does not permit a different NDP for a ligand in the vessel and that in the burette
97	MODE = 95 and MODE = 96 both apply

Section 11: MINIQUAD

This program accepts potentiometric data to refine estimates of formation constants for any equilibrium system applicable to program FORMAT excepting strong acid versus strong base reactions.

Keyword: MINIQ or MINI1

The program options are as follows.

IPROG
(Col 7)

n	- This digit corresponds to LARS in the main program: it causes every nth point in each titration to be included in the refinement. The default is 1.

JPROG
(Col 9)

1	- Causes a species distribution to be evaluated for the given formation constants and conditions.
2	- Gives maximum suppression of output
3	- Suppresses output from subroutine STATS only
4	- Generates STATS graphical output
5	- Sets the maximum number of iterations in the refinement cycle, MAXIT = 10
6	- Sets MAXIT = 20
7	- Sets MAXIT = 40
8	- Sets MAXIT = 80
9	- Sets MAXIT = 160

JPROG values between 5 and 9 should be unnecessary - use with caution. The default sets MAXIT = 50 when IPROG = 1 and MAXIT = 25 when IPROG 1.

The real number fields in cols 11-80 are ignored.

Miniquad reference: Talanta 1974, 21, 53

Section 12: MINIQUAD (75)

This program accepts potentiometric data to refine estimates of formation constants for any equilibrium system applicable to program FORMAT excepting strong acid versus strong base reactions.

Keyword: MINI2

The program options are the same as those for MINI1.

Miniquad (75) reference: Inorg.Chim.Acta 1976, 18, 237.

Section 13: SCOGS

This program accepts potentiometric data to refine estimates of formation constants for any equilibrium system applicable to program FORMAT excepting strong acid versus strong base reactions.

Keyword: SCOGS

The program options are as follows.

IPROG
(Col 7)

n - This digit corresponds to LARS in the main program; it causes every nth point in each titration to be included in the refinement. The default is 1.

JPROG
(Col 9)

n - This digit gives the number cycles to be calculated by the program (i.e. the number of cycles to be refined + 1). The default is 6.

A value of 1, therefore, causes a species distribution to be evaluated for the given formation constants and conditions.
A value of 2 causes one cycle of refinement and so on.

The other data fields are ignored.

SCOGS reference: Talanta 1968, 15, 1397

Section 14: COMICS

This program calculates the equilibrium concentrations of all species in solutions of metals and ligands at specified pH values. The total concentrations of the metal and ligand(s) must be provided for program FORMAT as well as the formation constant for each complex.

Keyword: COMIC

There are no program options.

COMICS reference: Talanta, 1967, 14, 833

Section 15: PSEUDOPLOT

This program calculates the equilibrium distribution of species in solutions of ligands and metal ions. It is primarily used to simulate titrations of such systems and to treat the simulated data to a ZPLOT analysis. Comparison of the PSEUDOPLOT with the experimental ZPLOT curves provides a stringent test of the formation constants selected to represent the equilibria in solution.

Keyword: PPLOT

The program options are as follows.

IPROG
(Col 7)

n — This digit corresponds to LARS in the MINIQUAD program: it causes every nth point in each titration to be included in the refinement. The default is 1 and IPROG=1 is used to include every odd point rather than every even one as would result from IPROG=2.

JPROG
(Col 9)

n — This digit controls the number of graphs to be drawn. With n=0 all titration curves are superimposed on one graph. With n=1 individual plots of each curve are drawn.

GRAPH
(Cols 11-20,
21-30,21-40 etc.

a,b,c,d,e,f,g — These seven parameters initiate the plotting routine and control the size of the graph.

a=maximum value for pA (or pH)
b=Minimum value for pA (or pH)
c=Length of pA (or pH) axis in cms (thesis size=22cm)
d=Maximum value for $\bar{Z}$
e=Minimum value for $\bar{Z}$
f=Length of $\bar{Z}$ axis in cms (thesis size=14cm)
g=size of characters on the plot in inches
(use 0.14 usually and 0.07 for smaller graphs)

PSEUDOPLOT reference: J.C.S.Dalton, 1975, 105.

Section 16: ZPLOT

This program calculates the $\bar{Z}$ function from experimental titration data. If there is no metal present in the solution curves of $\bar{Z}_H$ versus pH are generated (values of the protonation constants are not required for the calculation but dummy values should nevertheless be provided for program FORMAT.)
If there is metal present in the solution, curves of $\bar{Z}$ versus pH are produced (Values for the protonation constants are required and dummy values for some metal formation constants should also be provided.)

Keyword: ZPLOT

The program options are identical to those for PSEUDOPLOT except that JPROG (Col 9) is ignored.

ZPLOT reference: Rossotti,H.S. The Study of Ionic Equilibria, Longman, London, (1978) p.53.

Section 17: Dummy program

Section 18: ECCLES

This program calculates the equilibrium concentrations of all species in solutions of metals and ligands. The total or free concentrations of the components must be provided. Any such concentration can be scanned upwards from the initial value.

Keyword: ECCLS

There are no program options.

ECCLES reference: J.C.S.Dalton, 1977, 588.

Section 19: MAGEC

This program accepts potentiometric data for any equilibrium system applicable to program FORMAT excepting those which involve a metal ion. The primary use of this program is to optimise for the electrode parameters and equilibrium constants applicable to the system simultaneously. It can also be used to refine the concentrations of components in solution.

Keyword: MAGEC

The program options are as follows:

IPROG
(Col 7)

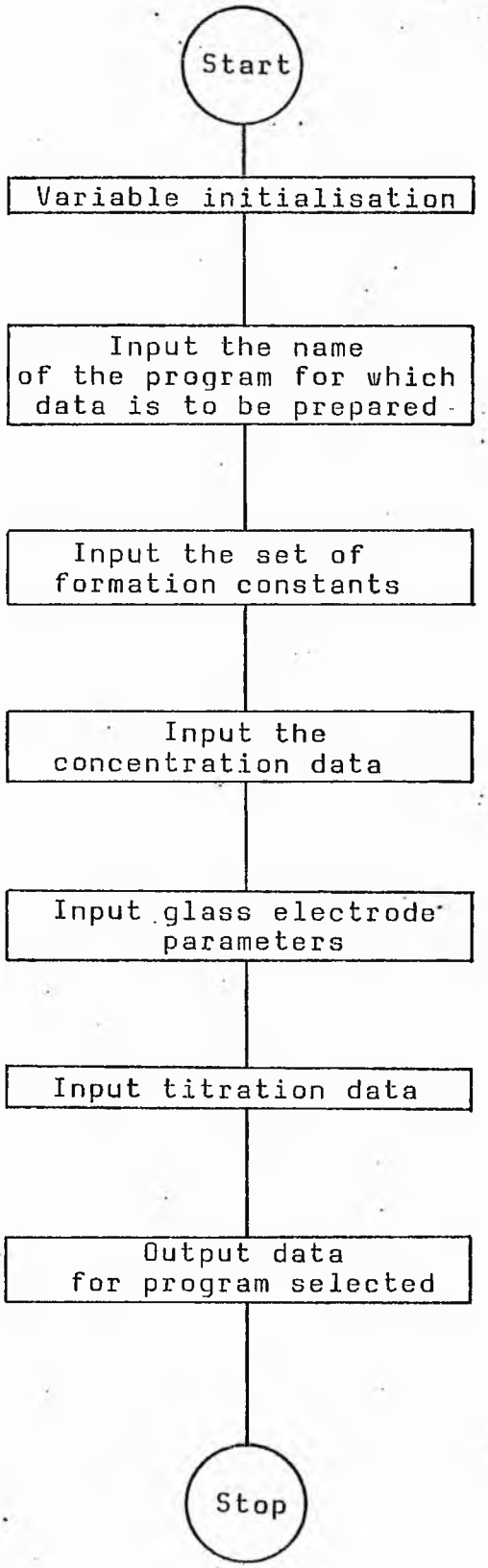
n — This digit corresponds to LARS in the MINIQUAD program: it causes every nth point in each titration to be included in the refinement. The default is 1 and IPROG=1 is used to include every odd point rather than every even one that would result from IPROG=2.

JPROG
(Col 9)

n — This digit specifies which of the titration data sets is to be prepared for program MAGEC. The default is to process all the data sets.

MAGEC reference: unpublished

FLOW DIAGRAM FOR PROGRAM FORMAT



```
C
C
C       PROGRAM FORMAT
C       --------------
C
C
C
C       DEVELOPED AT THE UNIVERSITY OF WALES INSTITUTE OF
                  SCIENCE AND
C       TECHNOLOGY DURING 1978.
C
C       THIS PROGRAM TRANSFORMS DATA PREPARED ACCORDING TO
                  A STANDARD
C       FORMAT INTO THE PARTICULAR FORMAT REQUIRED BY THE
                  MINIQUAD,
C       SCOGS, PSEUDOPLOT, ZPLOT, COMICS, ECCLES AND MAGEC
                  COMPUTER
C       PROGRAMS.
C
C       THE PROGRAM IS WRITTEN IN FORTRAN IV.
C
C
C
C
C
C       SECTION ONE:   STORAGE ALLOCATION AND FORMAT
                  STATEMENTS.
C
C
        INTEGER    PROGRM, OUTF, OUT, SEXHUN, ERROR
        INTEGER    TITLE(18), SPECIE(5), MATRIX(4,3),
                     REFINE(30)
        INTEGER    JQR(4,20), NDPV(2), NDPB(2), LIT(40),
                     SIHN(4)
        INTEGER    ALPHA(26), NUMBER(10), IDCHEK(20)
        DIMENSION    VALUE(3), VZERO(3), EZERO(3), SLOPE(3),
                     CONC(4)
        DIMENSION    BETA(3,20), CV(3,4), CB(3,4), GRAPH(7)
        DIMENSION  TITRE(100),  EMF(100)
        DOUBLE PRECISION  X, Y
C
C
        DATA IDCHEK  /'  PM','  DW','  I3','  CF','  BT','
                     TW',
       1  '  AC','  HA','  JD','  KQ','  CJ','  GS','  GK',
       2  'XXXX','XXXX','XXXX','XXXX','XXXX','XXXX','XXXX' /
C
C
        DATA LIT /'    ','MINI','SCOG','COMI','PPLO','ZPLO',
                     'NEWP',
       1    'ECCL','MAGE','CHEC','TITL','BETA','LBET','VESS',
                     'BURE',
       2    'CONC','GLAS','ML/M','ML/P','SCNP','INIT','ACID',
                     'LIG1',
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     3   'METL','LIG2','EZER','SLOP','TEMP','XXXX','FINI',
                 'H +1',
     4   'OH-1','XXXX','XXXX','XXXX','XXXX','XXXX','XXXX',
                 'XXXX','XXXX'/
      DATA  NUMBER / '1','2','3','4','5','6','7','8','9',
                 '0' /
      DATA  SIHN / ' ','+','-','*' /
      DATA  MATRIX / 2,4,3,1,2,3,1,0,2,1,0,0 /
      DATA  ALPHA /'A','B','C','D','E','F','G','H','I',
                 'J','K','L',
     1  'M','N','O','P','Q','R','S','T','U','V','W','X',
                 'Y','Z' /
C
C     THE PARAMETER VARIABLES
C
      IN = 5
      OUT = 6
      OUTF = 7
      MAXPTS = 100
      MAXTOT = 500
C
C     THE FORMAT STATEMENTS
C
10301 FORMAT(A4,A1,1X,I1,I2,1X,7G10.3)
10401 FORMAT(A4,A1,3X,18A4)
10511 FORMAT(5A1,1X,I1,3X,7G10.3)
10611 FORMAT(A4,A1,1X,I1,1X,A1,1X,7G10.3)
20000 FORMAT(18A4)
21201 FORMAT(16I5)
21211 FORMAT(F10.6,7I5)
21221 FORMAT(8F10.6)
21222 FORMAT(I5,8F8.3)
21311 FORMAT(40I2)
21321 FORMAT(5I2,F8.4)
21325 FORMAT(10F8.4)
21331 FORMAT(I2,F10.3)
21342 FORMAT(5F10.6,2F10.3)
21340 FORMAT(18A4,1X,'EXPT',I3)
21351 FORMAT(2F10.3,4X,I1)
21411 FORMAT(21I2,8X,F8.4)
21412 FORMAT(2I2,I3)
21430 FORMAT(10F8.6)
21452 FORMAT(F10.4,I1)
21511 FORMAT(25I3)
21531 FORMAT(7F10.4)
21534 FORMAT(13F6.3)
21542 FORMAT(I3,E11.4)
21550 FORMAT(I3,2X,'SIMULATION DATA PRODUCED BY PROGRAM
                 FORMAT')
21552 FORMAT(6E11.4)
21580 FORMAT(I3,2X,'TITRATION DATA PRODUCED BY PROGRAM
                 FORMAT')
21802 FORMAT('A SMALL EQUILIBRIUM SYSTEM',/
     1  'DATA PREPARED BY PROGRAM FORMAT')
21803 FORMAT('FALSE')
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21805 FORMAT('TRUE',2X,A4,1P2E10.4)
21806 FORMAT('MULTIPLY')
21810 FORMAT('TOTAL CONCENTRATIONS')
21812 FORMAT(A4,1X,1PE10.4)
21815 FORMAT('END')
21830 FORMAT('SPECIES CONSTANTS')
21835 FORMAT(F9.4,1X,5(A4,I3,2X))
21820 FORMAT('FREE CONCENTRATIONS')
21901 FORMAT('PK',A1,2X,I1,4X,1P3E10.3)
21921 FORMAT(3A1,2X,I1,4X,1P3E10.3)
21922 FORMAT('LG',A1,2X,I1,1X,I1,2X,1P3E10.3)
30000 FORMAT(////////'0',15X,35(1H*),8X,'THE
                  END',8X,35(1H*),//////)
30001 FORMAT('0')
30002 FORMAT('0','OUTPUT TO THE INTERMEDIATE DATA FILE')
30003 FORMAT('0','OUTPUT TO THE MAIN PROGRAM (',I1,').
                  COMPLETED.')
30004 FORMAT('0',I5,2X,'TITRATION DATA POINTS IN TOTAL')
30005 FORMAT('0','OUTPUT TO THE MAIN TO BE DELETED.')
30100 FORMAT('1',/'0',5(1H*),6X,3(1H*),6X,4(1H*),6X,1H*,
                  3X,1H*,
     1      6X,3(1H*),6X,5(1H*),/' ',1H*,9X,2(1H*,3X,1H*,
                  5X),
     2      2(2(1H*),1X),4X,1H*,3X,1H*,7X,1H*,/' ',
     3      4(1H*),6X,2(1H*,3X),2X,4(1H*),6X,3(1H*,1X),4X,
     4      5(1H*),7X,1H*,/' ',1H*,9X,2(1H*,3X),2X,1H*,2X,
     5      1H*,6X,2(2(1H*,3X),2X),2X,1H*,/' ',
     6      1H*,10X,3(1H*),6X,1H*,3X,1H*,2(2X,2(3X,1H*)),
                  7X,1H*,/' ',
     7      55(1H-),///////)
30101 FORMAT(//'0','SECTION',I3,/' ',10(1H-),/)
30102 FORMAT(' ','EXECUTION COMMENCED')
30201 FORMAT(' ','PROGRAM INITIALISED')
30304 FORMAT(' ','DATA PREPARATION FOR PROGRAM:')
30330 FORMAT('+',30X,'MINIQUAD')
30331 FORMAT('+',30X,'MINIQUAD(75)')
30332 FORMAT('+',30X,'SCOGS')
30333 FORMAT('+',30X,'COMICS')
30334 FORMAT('+',30X,'PSEUDOPLOT')
30335 FORMAT('+',30X,'ZPLOT')
30336 FORMAT('+',30X,'UNKNOWN')
30337 FORMAT('+',30X,'ECCLES')
30338 FORMAT('+',30X,'MAGEC')
30339 FORMAT('+',30X,'FORMAT ONLY')
30350 FORMAT('0','PROGRAM OPTIONS:',3X,2I4)
30351 FORMAT('0','NO PROGRAM OPTIONS')
30360 FORMAT('0','GRAPH OPTIONS:',3X,7(1PE15.4))
30450 FORMAT(' ','THE TITLE IS:',2X,18A4,/)
30460 FORMAT('0',20(1H*),5X,'IDENTIFICATION
                  ERROR',5X,20(1H*),/)
30521 FORMAT('0','LOGARITHM ERROR')
30541 FORMAT(' ','THERE ARE',I2,' COMPONENTS',/)
30545 FORMAT('0','UNRECOGNISABLE CHARACTER USED IN
                  DEFINING THE SPECIES:
     1   ',5A1)
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30555 FORMAT('0','MISPLACED SIGN')
30565 FORMAT('0','INCORRECT NUMBER OF COMPONENTS')
30567 FORMAT('0','FORMATION CONSTANTS',I3,'  AND',I3,'
                  REPRESENT DUPLIC
     1ATED SPECIES')
30570 FORMAT(' ','FORMATION CONSTANT LOG VALUE =',F8.3,'
                   FOR SPECIES',
     1  2X,5A1,2X,'TO BE ')
30572 FORMAT('+',85X,'WITH LIMITS',F8.3,'  AND  ',F8.3)
30573 FORMAT('+',67X,'REFINED')
30574 FORMAT('+',67X,'HELD CONSTANT')
30581 FORMAT(' ','FORMATION CONSTANT ERROR ON CARD
                  NUMBER',I4)
30591 FORMAT('0','NO FORMATION CONSTANT DATA
                  PROVIDED.',//)
30593 FORMAT('0','WATER DISSOCIATION CONSTANT DUPLICATED')
30596 FORMAT('0','UNREASONABLE ACID ASSOCIATION CONSTANT')
30598 FORMAT('0','NO PROTONATION CONSTANTS PROVIDED.',/)
30605 FORMAT('0','CONCENTRATION DATA PROBABLY STARTED
                  PREMATURELY')
30609 FORMAT(' ',74X,5(1H-),3X,A4,A1,1X,'INPUT',3X,5(1H-))
30610 FORMAT('+',108X,'STARTS AT CARD NO.',I4)
30611 FORMAT(' ','REFINE THE VALUE FOR  ',A4,A1)
30612 FORMAT('+',30X,'BETWEEN THE LIMITS',1PE12.4,'
                  AND ',1PE12.4)
30613 FORMAT('0','THE ESTIMATE DOES NOT LIE BETWEEN THE
                  LOWER AND UPPER
     1LIMITS, WHICH MUST BE SPECIFIED IN THAT ORDER.')
30614 FORMAT(' ','SCAN THE VALUE FOR  ',A4,A1)
30622 FORMAT(' ','THE INITIAL VOLUME IN THE VESSEL
                  IS;',F7.2)
30631 FORMAT('0','NO FORMATION CONSTANT DATA FOR
                  COMPONENT',1X,A4)
30634 FORMAT('0','MISSING COMPONENT',1X,A4)
30636 FORMAT('0','NO COMPONENTS IN VESSEL AND/OR BURETTE')
30639 FORMAT('0THERE IS NO VALUE FOR THE INITIAL VOLUME
                  IN THE VESSEL')
30644 FORMAT('0',38X,A4,4X,1PE15.4)
30645 FORMAT('+',30X,'FREE')
30646 FORMAT('+',30X,'TOTAL')
30648 FORMAT(///'0THE EQUILIBRIUM CONCENTRATIONS ARE TO
                  BE CALCULATED',/
     1    ' ','AT EACH OF THE FOLLOWING NEGATIVE LOG
                  VALUES FOR ',A4,///)
30649 FORMAT(' ',30X,F10.3)
30650 FORMAT(///'0','THE COMPONENT CONCENTRATIONS
                  ARE:',//'0',
     1   26X,4(A4,11X))
30651 FORMAT(/'0',10X,'VESSEL',4X,4(1PE15.4))
30652 FORMAT('0',10X,'BURETTE',3X,4(1PE15.4))
30655 FORMAT('0',A4,A1,'  INPUT DATA DUPLICATED')
30656 FORMAT('0','PARITY CHECK ON 5TH LETTER OF KEYWORD
                  FAILS')
30658 FORMAT('0','UNREASONABLE VALUE PROVIDED FOR ',A4,A1)
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30661 FORMAT(' ','THE NUMBER OF DISSOCIABLE PROTONS FROM
                  ',A4,
     1  '  IN THE VESSEL:',I3)
30662 FORMAT('0','NDP COLUMN IS BLANK')
30663 FORMAT('0','INVALID NDP ENTRY FOR ',A4,A1)
30666 FORMAT(' ','THE NUMBER OF DISSOCIABLE PROTONS FROM
                  ',A4,
     1  '  IN THE BURETTE:',I3)
30670 FORMAT('0','ONLY ONE SCAN COMPONENT PERMITTED.')
30671 FORMAT(' ',A4,1X,'HAS',I2,1X,'DISSOCIABLE PROTONS')
30722 FORMAT(' ','THE INTERCEPT OF THE ELECTRODE RESPONSE
                  IS:',F7.2)
30733 FORMAT(' ','THE SLOPE OF THE ELECTRODE RESPONSE
                  IS:',F7.2)
30740 FORMAT(' ','THE TEMPERATURE IS:',F6.2,
     1    '  (USED TO CALCULATE THE NERNSTIAN SLOPE)')
30745 FORMAT('0','TEMPERATURE MUST FOLLOW A VALUE FOR
                  EZERO.',//)
30750 FORMAT('0','GLASS ELECTRODE PARAMETER MISSING')
30752 FORMAT(' ','ARBITARY POSITIVE VALUE SUPPLIED',//)
30801 FORMAT('0','TITRATION DATA INPUT BEGINS.',
     1  10X,'TITRATION NUMBER',I3,//)
30802 FORMAT('0',10X,'CARD NO.',12X,'VOL.',5X,A4,A1,/)
30850 FORMAT(///'0TITRATION DATA STORAGE BUFFER CAPACITY
                  EXCEEDED',////)
30855 FORMAT(////'0','SUBSEQUENT CARDS ARE IGNORED UNTIL
                  A RECOGNISABLE
     1KEYWORD IS ENCOUNTERED',///)
30870 FORMAT(//'0','TITRATION DATA INPUT COMPLETED:',10X,
     1   I3,' POINTS REGISTERED IN CORE.')
30872 FORMAT('0',I5,' DATA POINTS EXCEED THE PERMITTED
                  LIMIT OF',I5,//)
30880 FORMAT('0','POINT NUMBER',I4,3X,'(',F7.3,'
                  ,',F8.2,' )',
     1  '  CORRESPONDS TO AN UNREASONABLE PH
                  =',1PE12.3,//)
30881 FORMAT('+',59X,', BUT NOT NECESSARILY IN ERROR.')
30882 FORMAT('0','POINT NUMBER',I4,3X,'(',F7.3,'
                  ,',F8.2,' )',
     1  '  IS OUT OF SEQUENCE')
30883 FORMAT('+',99X,'CHECK THE PREVIOUS POINT.')
30884 FORMAT('0','ASSUME THERE HAS BEEN A SWITCH IN
                  DIRECTION ...',//)
30885 FORMAT('0','PROBABLY DUE TO AN AN ERROR IN',
     1 ' THE FIRST AND/OR SECOND DATA POINT.')
30887 FORMAT('0','THE TITRATION DATA AND THE ELECTRODE
                  PARAMETERS ARE IN
     1COMPATIBLE',///)
30888 FORMAT(' ','ELECTRODE PARAMETERS PROBABLY IN
                  ERROR',//)
30901 FORMAT(' ','INPUT TERMINATED')
31001 FORMAT('0','INCOMPLETE DATA CAUSES PROGRAM
                  TERMINATION')
31002 FORMAT('0','WHERE? OH, WHERE IS YOUR INPUT DATA?')
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```
31010 FORMAT('0',A4,A1,2X,'IS AN UNRECOGNISABLE OR
                    MISPLACED KEYWORD')
31011 FORMAT('0','THE KEYWORD ENTRY IS BLANK')
31051 FORMAT('0','ERROR DETECTED ON CARD NUMBER',I4)
31052 FORMAT('+',70X,'THE CARD IS IGNORED.')
31077 FORMAT('0','SEQUENCE ERROR ON CARD NUMBER',I4)
31091 FORMAT(/'0','THE CURRENT')
31092 FORMAT(/'0','THE  FINAL')
31093 FORMAT('+',12X,'TOTAL FOR ERRORS DETECTED ON INPUT
                    =',I3)
31097 FORMAT(' ','HAS PROVED IMPOSSIBLE.',/' ',
     1    'AN UNSUITABLE SYSTEM HAS BEEN DEFINED.',/' ',
     2    'MAIN PROGRAM EXECUTION IS TERMINATED.',/' ',
     3    'ERROR MODE CODE =',I4)
31099 FORMAT(///'0','IF YOU HAD TO RELY ON THIS FOR A
                    LIVING',/' ',
     1  'YOU WOULD SURELY STARVE!',///)
31901 FORMAT('0','TITRATION DATA SET IGNORED')
C
C
      WRITE(OUT,30100)
      SEXHUN = 1
      WRITE(OUT,30101)  SEXHUN
      WRITE(OUT,30102)
C
C
C
C     SECTION TWO:   INITIALISATION.
C
C
      SEXHUN = 2
      WRITE(OUT,30101)  SEXHUN
      ERROR = 0
      NNN = 0
      NFLAG = 0
      NTITR = 0
      ISCN = 0
      IGRAPH = 0
      IKW = 0
      NP = 0
      NPTOT = 0
      MODE = 0
      NDPSTR = -1
      SCNINC = 0.000
      SCNMAX = 0.000
      DO 201 I=1,20
      DO 201 J=1,4
  201 JQR(J,I) = 0
      DO 202 I=1,4
  202 CONC(I) = 0.000
      DO 205 I=1,30
  205 REFINE(I) = -1
      WRITE(OUT,30201)
C
C
```

```
C
C       SECTION THREE;    PROGRAM KEYWORDS.
C
C
  300 SEXHUN = 3
      WRITE(OUT,30101)  SEXHUN
  301 NNN = NNN + 1
      READ(IN,10301,END=1000,ERR=1050)  KEY1, KEY2, IVAL,
                    JVAL, GRAPH
  304 WRITE(OUT,30304)
      I = SEXHUN - 10
      IF(I.GT.0)  GO TO 320
      DO 310 I=2,10
      IF(KEY1.EQ.LIT(I))  GO TO 320
  310 CONTINUE
      GO TO 1010
  320 PROGRM = I
      IPROG = IVAL
      JPROG = JVAL
      GO TO (331,331,332,333,334,335,336,337,338,339),
                    PROGRM
  331 IF(KEY2.EQ.NUMBER(1).OR.KEY2.EQ.ALPHA(17))
                    PROGRM = 1
      IF(PROGRM.EQ.1)  WRITE(OUT,30330)
      IF(PROGRM.EQ.2)  WRITE(OUT,30331)
      IF(IPROG.GT.1)  MAXTOT = MAXTOT * IPROG
      GO TO 350
  332 WRITE(OUT,30332)
      GO TO 350
  333 WRITE(OUT,30333)
      GO TO 350
  334 WRITE(OUT,30334)
      GO TO 350
  335 WRITE(OUT,30335)
      GO TO 350
  336 WRITE(OUT,30336)
      GO TO 350
  337 WRITE(OUT,30337)
      GO TO 350
  338 WRITE(OUT,30338)
      GO TO 350
  339 WRITE(OUT,30339)
C
  350 IF(SEXHUN.GT.4)  GO TO 1092
      IF(IPROG+JPROG.EQ.0)  GO TO 351
      WRITE(OUT,30350)  IPROG, JPROG
      GO TO 352
  351 WRITE(OUT,30351)
  352 DO 353 I=1,7
      IF(GRAPH(I).GT.0.000)  GO TO 360
  353 CONTINUE
      GO TO 400
  360 WRITE(OUT,30360)  GRAPH
      IGRAPH = 1
C
```

```
C
C
C     SECTION FOUR:   TITLE KEYWORD.
C
C
  400 SEXHUN = 4
      WRITE(OUT,30101)  SEXHUN
  401 NNN = NNN + 1
      READ(IN,10401,END=1000,ERR=1050)   KEY1, KEY2, TITLE
      IF(KEY1.NE.LIT(11))  GO TO 1010
      WRITE(OUT,30450)  TITLE
C
      DO 450 I=1,20
      IF(TITLE(18).EQ.IDCHEK(I)) GO TO 500
  450 CONTINUE
      ERROR = ERROR + 1
      WRITE(OUT,30460)
C
C
C
C     SECTION FIVE:   FORMATION CONSTANTS.
C
C
  500 SEXHUN = 5
      WRITE(OUT,30101)  SEXHUN
  501 NNN = NNN + 1
      READ(IN,10301,END=1000,ERR=1050)  KEY1, KEY2
      IF(KEY1.NE.LIT(12).AND.KEY1.NE.LIT(13))  GO TO 1010
  505 NS = 0
      NC = -1
      NREF = 0
C
  510 NS = NS + 1
      NEWERR = ERROR
  511 NNN = NNN + 1
      READ(IN,10511,END=1000,ERR=580)   SPECIE, IVAL,
                  VALUE
      IF(SPECIE(1).EQ.ALPHA(22).OR.SPECIE(1).EQ.ALPHA(3))
                   GO TO 590
      REFINE(NS) = IVAL
C
C     LOAD THE FORMATION CONSTANTS, TAKING LOGS IF
                  NECESSARY.
C
  520 IF(KEY1.EQ.LIT(13))  GO TO 525
      IF(VALUE(1).GT.0.000)  GO TO 522
  521 WRITE(OUT,30521)
      KEY1 = LIT(13)
      GO TO 580
  522 VALUE(1) = ALOG10(VALUE(1))
      IF(IVAL.NE.2)  GO TO 525
      IF(VALUE(2).LE.0.000.OR.VALUE(3).LE.0.000)
                   GO TO 521
      VALUE(2) = ALOG10(VALUE(2))
      VALUE(3) = ALOG10(VALUE(3))
```

```
      IF(VALUE(1).GE.VALUE(2).AND.VALUE(1).LE.VALUE(3))
                    GO TO 525
      WRITE(OUT,30613)
      ERROR = ERROR + 1
  525 DO 526 I=1,3
  526 BETA(I,NS) = VALUE(I)
C
C     SET NC = NUMBER OF COMPONENTS.
C
  530 IF(NC.GT.0)  GO TO 540
      DO 535 NC=1,5
      IF(SPECIE(NC).EQ.SIHN(1))  GO TO 538
  535 CONTINUE
      NC = 6
  538 NC = NC - 2
      IF(NC.GE.1)  GO TO 539
      NC = -1
      GO TO 565
  539 IF(SPECIE(NC).EQ.SIHN(2).OR.SPECIE(NC).EQ.SIHN(3))
                    NC = NC - 1
      NC = NC + 1
      N = 5 - NC
      WRITE(OUT,30541)  NC
      IF(NC.GT.1.AND.NC.LT.5)  GO TO 540
      WRITE(OUT,30565)
      ERROR = ERROR + 1
      IF(NC.LT.2)  NC = 2
      IF(NC.GT.4)  NC = 4
C
C     LOAD THE JQR MATRIX WITH SPECIES' COMPONENT
                    MULTIPLICITIES.
C
  540 I = 0
      M = 1
      K = 0
  543 I = I + 1
      IF(SPECIE(I).EQ.SIHN(1).OR.I.EQ.6)  GO TO 560
      IF(SPECIE(I).EQ.SIHN(2))  GO TO 555
      IF(SPECIE(I).EQ.SIHN(3))  GO TO 550
      DO 545 J=1,10
      IF(SPECIE(I).EQ.NUMBER(J))  GO TO 548
  545 CONTINUE
      IF(NEWERR.GT.ERROR)  GO TO 1055
      NEWERR = ERROR + 2
      WRITE(OUT,30545)  SPECIE
      GO TO 580
  548 K = K + 1
      IF(K.GT.NC)  GO TO 565
      L = MATRIX(K,N)
      IF(J.EQ.10)  J = 0
      JQR(L,NS) = J * M
      GO TO 543
C
C     MAKE THE HYDROGEN ION MULTIPLICITY NEGATIVE.
C
```

```
  550 M = -1
  555 IF(I.EQ.NC)  GO TO 543
      WRITE(OUT,30555)
      GO TO 580
C
C     CHECK THE NUMBER OF COMPONENTS IN THE SPECIES.
C
  560 IF(K.EQ.NC)  GO TO 566
  565 WRITE(OUT,30565)
      IF(NS.EQ.2.OR.NEWERR.GT.ERROR)  NC = -1
      NEWERR = NEWERR + 2
      GO TO 580
C
C     CHECK FOR SPECIES DUPLICATION.
C
  566 IF(NS.EQ.1)  GO TO 570
      JVAL = NS - 1
      DO 568 I=1,JVAL
      DO 567 L=1,NC
      IF(JQR(L,I).NE.JQR(L,NS))  GO TO 568
  567 CONTINUE
      ERROR = ERROR + 1
      WRITE(OUT,30567)  I, NS
  568 CONTINUE
C
C     PRINT THE FORMATION CONSTANT.
C
  570 WRITE(OUT,30570)  BETA(1,NS),  (SPECIE(I), I=1,5)
      IF(JQR(1,NS).EQ.-1.AND.JQR(2,NS).EQ.0.AND.JQR(3,
                  NS).EQ.0
     1   .AND.JQR(4,NS).EQ.0)  IKW = IKW + NS
      IF(IVAL-1)  574, 573, 572
  572 WRITE(OUT,30572)  BETA(2,NS), BETA(3,NS)
  573 WRITE(OUT,30573)
      NREF = NREF + 1
      GO TO 510
  574 WRITE(OUT,30574)
      GO TO 510
C
C     ERROR DETECTED AMONGST THE FORMATION CONSTANTS.
C
  580 ERROR = ERROR + 1
      WRITE(OUT,30581)  NNN
      IF(ERROR.GT.20)  GO TO 1090
      GO TO 511
C
C     TEST FOR STRONG ACID / STRONG BASE TITRATION.
C
  590 NS = NS - 1
      IF(NS.EQ.1.AND.JQR(2,1).EQ.0.AND.NC.EQ.2)  NC = 1
      IF(NC.GT.0)  GO TO 593
      ERROR = ERROR + 2
      WRITE(OUT,30591)
  593 IF(IKW.LE.NS)  GO TO 594
      WRITE(OUT,30593)
```

```
      ERROR = ERROR + 1
  594 PKW = 14.00
      IF(IKW.LT.1)  GO TO 600
      PKW = -BETA(1,IKW)
C
C     TEST FOR PROTONATION CONSTANTS.
C
      M = 0
      IF(NC.LE.1.OR.NC.GT.3)  GO TO 600
      DO 598 I=1,NS
      IF(I.EQ.IKW.OR.(NC.EQ.3.AND.JQR(3,I).NE.0))
                  GO TO 598
      IF(M.LT.-900)  GO TO 596
      M = M + 1
      IF(BETA(1,I)*JQR(1,I).GT.0.000001)  GO TO 598
      IF(M.NE.1)  GO TO 596
      M = -1000
      GO TO 598
  596 ERROR = ERROR + 1
      IF(M.LT.-900)  M = -500
      WRITE(OUT,30596)
  598 CONTINUE
      IF(M.NE.0)  GO TO 600
      ERROR = ERROR + 1
      WRITE(OUT,30598)
C
C
C
C     SECTION SIX:   CONCENTRATION DATA.
C
C
  600 SEXHUN = 6
      WRITE(OUT,30101)  SEXHUN
      NEWERR = ERROR
      DO 602 I=21,30
  602 REFINE(I) = -1
      DO 603 I=1,3
      VZERO(I) = 0.000
      EZERO(I) = 0.000
      SLOPE(I) = 0.000
      DO 603 J=1,4
      CV(I,J) = 0.000
  603 CB(I,J) = 0.000
      DO 604 I=1,2
      NDPV(I) = -1
  604 NDPB(I) = -1
      EMF(1) = 0.000
      EMF(2) = 0.000
      KEY1 = LIT(1)
      IF(SPECIE(1).EQ.ALPHA(22).AND.SPECIE(2).EQ.ALPHA(5)
                  .AND.SPECIE(3)
     1    .EQ.ALPHA(19).AND.SPECIE(4).EQ.ALPHA(19))
                  KEY1 = LIT(14)
      IF(SPECIE(1).EQ.ALPHA(3).AND.SPECIE(2).EQ.ALPHA(15)
                  .AND.SPECIE(3)
```

```
     1    .EQ.ALPHA(14).AND.SPECIE(4).EQ.ALPHA(3))
                    KEY1 = LIT(16)
   605 MODE = -1
       IF(KEY1.EQ.LIT(14))  MODE = 1
       IF(KEY1.EQ.LIT(15))  MODE = 2
       IF(KEY1.EQ.LIT(16))  MODE = 3
       IF(MODE-2)  606, 607, 608
   606 K = ALPHA(12)
       IF(MODE.GT.0)  GO TO 609
       WRITE(OUT,30605)
   607 K = ALPHA(20)
       GO TO 609
   608 K = ALPHA(19)
   609 WRITE(OUT,30609)  KEY1, K
       WRITE(OUT,30610)  NNN
C
C
C      REFINEMENT KEY
C
C      REFINE(1) - REFINE(20)    FOR BETA
C      REFINE(21)   FOR   VZERO
C      REFINE(22)   FOR   ACIDV   OR   ACIDT/ACIDF
C      REFINE(23)   FOR   LIG1V   OR   LIG1T/LIG1F
C      REFINE(24)   FOR   METLV   OR   METLT/METLF
C      REFINE(25)   FOR   LIG2V   OR   LIG2T/LIG2F
C      REFINE(26)   FOR   ACIDB
C      REFINE(27)   FOR   LIG1B
C      REFINE(28)   FOR   METLB
C      REFINE(29)   FOR   LIG2B   OR   EZERO
C      REFINE(30)   FOR   TEMP    OR   SLOPE
C
C
   610 NNN = NNN + 1
       READ(IN,10611,END=659,ERR=657)  KEY1, KEY2, IVAL,
                    JVAL, VALUE
       IF(IVAL.EQ.1.OR.IVAL.EQ.2)  WRITE(OUT,30611)
                    KEY1, KEY2
       IF(IVAL.EQ.3.OR.IVAL.EQ.4)  WRITE(OUT,30614)
                    KEY1, KEY2
       DO 611 I=1,9
       IF(JVAL.EQ.NUMBER(I))  GO TO 612
   611 CONTINUE
       I = 0
       IF(JVAL.EQ.NUMBER(10))  GO TO 612
       I = -1
       IF(JVAL.NE.SIHN(1))  GO TO 663
   612 JVAL = I
       K = 0
       IF(IVAL.NE.2)  GO TO 613
       WRITE(OUT,30612)  VALUE(2), VALUE(3)
       IF(VALUE(1).GE.VALUE(2).AND.VALUE(1).LE.VALUE(3))
                    GO TO 613
       WRITE(OUT,30613)
       ERROR = ERROR + 1
   613 IF(KEY1.NE.LIT(22).AND.VALUE(1).LT.1.0E-20)  K = 1
```

```
  614 DO 615 I=22,25
      IF(KEY1.EQ.LIT(I))  GO TO 618
  615 CONTINUE
      IF(MODE-2)  620, 630, 640
  618 J = I - 21
      IF(K.EQ.1)  GO TO 658
      IF(MODE-2)  660, 665, 670
C
C     LOAD THE VALUE FOR THE INITIAL VOLUME.
C
  620 IF(KEY1.NE.LIT(21))  GO TO 625
      IF(K.EQ.1)  GO TO 658
      IF(KEY2.NE.ALPHA(22))  GO TO 656
      IF(REFINE(21).NE.-1)  GO TO 655
      DO 621 I=1,3
  621 VZERO(I) = VALUE(I)
      REFINE(21) = IVAL
      WRITE(OUT,30622)  VZERO(1)
      GO TO 610
  625 IF(KEY1.NE.LIT(15))  GO TO 1010
      MODE = 2
      GO TO 607
C
C
C     TITRATION MODE EXIT CHECKING PROCEDURES.
C
C
C     CHECK THAT THERE ARE NOT TOO MANY COMPONENTS.
C
C
  630 IF(ERROR.GT.0.AND.ERROR.NE.NEWERR)  GO TO 650
      WRITE(OUT,30609)  LIT(10), ALPHA(11)
      IF(KEY1.NE.LIT(17).AND.KEY1.NE.LIT(18)
                  .AND.KEY1.NE.LIT(19))
     1   GO TO  1010
      IF(NC-3)  631, 632, 634
  631 IF(NC.EQ.1)  GO TO 637
      IF(NC.LT.1.AND.REFINE(23)+REFINE(27).NE.-2)
                  WRITE(OUT,30631)LIT(23)
      IF(REFINE(24)+REFINE(28).EQ.-2.OR.NC.EQ.2)
                  GO TO 632
      ERROR = ERROR + 1
      NC = 3
      WRITE(OUT,30631)  LIT(24)
  632 IF(REFINE(25)+REFINE(29).EQ.-2)  GO TO 635
      ERROR = ERROR + 1
      NC = 4
      WRITE(OUT,30631)  LIT(25)
C
C     CHECK THAT THERE ARE NOT TOO FEW COMPONENTS.
C
  634 IF(REFINE(25)+REFINE(29).GT.-2)  GO TO 635
      ERROR = ERROR + 1
      WRITE(OUT,30634)  LIT(25)
```

```
  635 IF(NC.EQ.2.OR.REFINE(24)+REFINE(28).GT.-2)
                  GO TO 636
      ERROR = ERROR + 1
      WRITE(OUT,30634)  LIT(24)
  636 IF(REFINE(23)+REFINE(27).GT.-2)  GO TO 637
      ERROR = ERROR + 1
      WRITE(OUT,30634)  LIT(23)
C
C     CHECK THAT THE COMPONENTS EXIST IN REALISTIC
                  CONCENTRATIONS.
C
  637 SUMV = -1.0E-18
      SUMB = -1.0E-18
      DO 638 I=1,NC
      SUMV = SUMV + ABS(CV(1,I))
  638 SUMB = SUMB + ABS(CB(1,I))
      IF(SUMV.GT.0.000.AND.SUMB.GT.0.000)  GO TO 639
      ERROR = ERROR + 1
      WRITE(OUT,30636)
C
C     CHECK THAT THE INITIAL VOLUME IS KNOWN.
C
  639 IF(REFINE(21).NE.-1)  GO TO 650
      WRITE(OUT,30639)
      ERROR = ERROR + 1
      GO TO 650
C
C
C     SIMULATION MODE EXIT PROCEDURES.
C
C
C      DEAL WITH SCNPH OR SCNPA.   (EMF = -LOG(CONC))
C
C
  640 IF(KEY1.EQ.LIT(30))  GO TO 643
      IF(KEY1.NE.LIT(20))  GO TO 1010
      IF(ISCN.NE.0)  GO TO 671
      IVAL = 0
      IF(KEY2.EQ.ALPHA(8))  IVAL = 1
      IF(KEY2.EQ.ALPHA(1))  IVAL = 2
      IF(IVAL.EQ.0)  GO TO 656
      J = IVAL + 21
      IF(REFINE(J).NE.-1.AND.REFINE(J).NE.10)  GO TO 671
      REFINE(J) = 10
      NP = NP + 1
      EMF(NP) = VALUE(1)
      GO TO 610
C
  643 IF(ISCN.NE.0)  GO TO 645
      IF(REFINE(22).EQ.10)  ISCN = 1
      IF(REFINE(23).EQ.10)  ISCN = 2
      IF(ISCN.EQ.0)  GO TO 645
      A = -EMF(1)
      CONC(ISCN) = 10.000 ** A
C
```

```
C     PRINT OUT THE CONCENTRATION DATA (SIMULATIONS).
C
  645 NFLAG = 2
      DO 647 I=1,NC
      J = I + 21
      WRITE(OUT,30644)  LIT(J), CONC(I)
      IF(REFINE(J).EQ.1)  GO TO 646
      WRITE(OUT,30645)
      GO TO 647
  646 WRITE(OUT,30646)
  647 CONTINUE
      IF(NP.LE.1)  GO TO 900
      J = ISCN + 21
      WRITE(OUT,30648)  LIT(J)
      DO 649 I=1,NP
  649 WRITE(OUT,30649)  EMF(I)
      GO TO 900
C
C     PRINT OUT THE CONCENTRATION DATA (TITRATIONS).
C
  650 K = NC + 21
      WRITE(OUT,30650)  (LIT(I), I=22,K)
      WRITE(OUT,30651)  (CV(1,I), I=1,NC)
      WRITE(OUT,30652)  (CB(1,I), I=1,NC)
      WRITE(OUT,30001)
      GO TO 700
C
C     ERRORS DETECTED IN THE CONCENTRATION DATA.
C
  655 WRITE(OUT,30655)  KEY1, KEY2
      GO TO 657
  656 WRITE(OUT,30656)
  657 ERROR = ERROR + 1
      WRITE(OUT,31051)  NNN
      WRITE(OUT,30001)
      GO TO 610
  658 K = 0
      WRITE(OUT,30658)  KEY1, KEY2
      ERROR = ERROR + 1
      GO TO 614
  659 IF(MODE.EQ.3)  GO TO 645
      GO TO 1000
C
C     SET CONCENTRATION VALUES FOR THE TITRATION.
C
  660 IF(KEY2.NE.ALPHA(22))  GO TO 656
      IF(REFINE(I).NE.-1)  GO TO 655
      REFINE(I) = IVAL
      DO 661 I=1,3
  661 CV(I,J) = VALUE(I)
      IF(J.NE.2.AND.J.NE.4)  GO TO 610
      IF(JVAL.LT.0)  GO TO 662
      J = J / 2
      NDPV(J) = JVAL
      WRITE(OUT,30661)  KEY1, JVAL
```

```
      GO TO 610
  662 WRITE(OUT,30662)
  663 WRITE(OUT,30663)  KEY1, KEY2
      GO TO 657
  665 I = I + 4
      IF(KEY2.NE.ALPHA(2))  GO TO 656
      IF(REFINE(I).NE.-1)  GO TO 655
      REFINE(I) = IVAL
      DO 666 I=1,3
  666 CB(I,J) = VALUE(I)
      IF(J.NE.2.AND.J.NE.4)  GO TO 610
      IF(JVAL.LT.0)  GO TO 662
      J = J / 2
      NDPB(J) = JVAL
      WRITE(OUT,30666)  KEY1, JVAL
      GO TO 610
C
C     SET CONCENTRATION VALUES FOR THE EQUILIBRIUM
                DISTRIBUTION CALC.
C
  670 IF(REFINE(I).NE.-1)  GO TO 655
      CONC(J) = VALUE(1)
      IF(IVAL.EQ.0)  GO TO 673
      IF(ISCN.EQ.0)  GO TO 672
  671 WRITE(OUT,30670)
      GO TO 657
  672 IF(IVAL.GT.0)  ISCN = J
      IF(IVAL.EQ.4)  ISCN = -J
      SCNMAX = VALUE(2)
      SCNINC = VALUE(3)
      IVAL = 0
  673 IF(KEY2.EQ.ALPHA(20))  IVAL = 1
      IF(KEY2.EQ.ALPHA(6))  IVAL = 2
      IF(IVAL.EQ.0)  GO TO 656
      REFINE(I) = IVAL
      IF(J.NE.2.AND.J.NE.4)  GO TO 610
      J = J / 2
      NDPV(J) = JVAL
      WRITE(OUT,30671)  KEY1, JVAL
      GO TO 610
C
C
C
C      SECTION SEVEN:   GLASS ELECTRODE PARAMETERS.
C
C
  700 IF(KEY1.NE.LIT(17))  GO TO 800
      SEXHUN = 7
      WRITE(OUT,30101)  SEXHUN
C
  710 NNN = NNN + 1
      READ(IN,10301,END=1000,ERR=1050)  KEY1, KEY2, IVAL,
                JVAL, VALUE
      IF(IVAL.GT.0)  WRITE(OUT,30611)  KEY1, KEY2
      IF(IVAL.EQ.2)  WRITE(OUT,30612)  VALUE(2), VALUE(3)
```

```
      DO 715 I=26,28
      IF(KEY1.EQ.LIT(I))  GO TO 718
  715 CONTINUE
      IF(KEY1.EQ.LIT(18).OR.KEY1.EQ.LIT(19))  GO TO 750
      GO TO 1010
  718 IF(I-27)  720, 730, 740
C
  719 ERROR = ERROR + 1
      WRITE(OUT,30655)  KEY1, KEY2
      WRITE(OUT,31051)  NNN
      GO TO 710
C
  720 IF(REFINE(29).NE.-1)  GO TO 719
      REFINE(29) = IVAL
      DO 722 I=1,3
  722 EZERO(I) = VALUE(I)
      WRITE(OUT,30722)  VALUE(1)
      GO TO 710
C
  730 IF(REFINE(30).NE.-1)  GO TO 719
      REFINE(30) = IVAL
      DO 733 I=1,3
  733 SLOPE(I) = VALUE(I)
      WRITE(OUT,30733)  VALUE(1)
      GO TO 710
C
  740 WRITE(OUT,30740)  VALUE(1)
      IF(REFINE(29).LT.0)  EZERO(1) = 400.0
      DO 741 I=1,3
  741 VALUE(I) = SIGN(((VALUE(I)+273.15)*0.1984161),
                      EZERO(1))
      KEY1 = LIT(27)
      KEY2 = ALPHA(5)
      IF(REFINE(29).GT.-1)  GO TO 730
      WRITE(OUT,30745)
      WRITE(OUT,30752)
      ERROR = ERROR + 1
      GO TO 730
C
  750 IF(REFINE(29).GT.-1.AND.REFINE(30).GT.-1)  GO TO 800
      WRITE(OUT,30750)
      ERROR = ERROR + 1
      IF(REFINE(29).LT.0)  EZERO(1) = 400.0
      IF(REFINE(30).LT.0) SLOPE(1) = SIGN(60.0,EZERO(1))
      WRITE(OUT,30752)
C
C
C
C     SECTION EIGHT:   TITRATION DATA INPUT.
C
C
  800 SEXHUN = 8
      WRITE(OUT,30101)  SEXHUN
  801 NP = 0
      K = 1
```

```
      IF(IPROG.EQ.1)  K = 0
      L = IPROG
      IF(IPROG.EQ.1)  L = 2
      NTITR = NTITR + 1
      WRITE(OUT,30801)  NTITR
      WRITE(OUT,30802)  KEY1, KEY2
      NEWERR = ERROR + 1
      NFLAG = 0
      GO TO 803
  802 IF(NEWERR.EQ.ERROR)  GO TO 855
      ERROR = ERROR + 1
      IF(ERROR.GT.20)  GO TO 1090
      NFLAG = 0
  803 NNN = NNN + 1
      K = K + 1
      CALL FREED(X,Y,NNN,NFLAG,IN,OUT,NUMBER,ALPHA(6),
                 ALPHA(22),SPECIE)
      IF(NFLAG)  802, 804, 870
  804 NEWERR = ERROR + 2
  805 IF(IPROG.EQ.0)  GO TO 806
      IF(MOD(K,L).NE.0)  GO TO 803
  806 NP = NP + 1
      IF(MAXPTS-NP+1)  803, 850, 807
  807 TITRE(NP) = X
      EMF(NP) = Y
      GO TO 803
C
C     ERROR PROCESSOR.
C
  850 WRITE(OUT,30850)
      ERROR = ERROR + 1
      GO TO 803
  855 WRITE(OUT,30855)
      ERROR = ERROR + 1
  856 READ(IN,10301,END=1000,ERR=1050)  KEY1, KEY2
      DO 857 I=2,30
      IF(KEY1.EQ.LIT(I))  GO TO 1060
  857 CONTINUE
      GO TO 856
C
C     TITRATION DATA CHECKING PROCEDURES.
C
  870 IF(NP.GT.MAXPTS)  NP = MAXPTS
      WRITE(OUT,30870)  NP
      K = 0
      NPTOT = NPTOT + NP
      IF(NPTOT.LE.MAXTOT)  GO TO 872
      WRITE(OUT,30872)  NPTOT, MAXTOT
      ERROR = ERROR + 1
      NPTOT = -100000
  872 IF(KEY1.EQ.LIT(18))  GO TO 880
      DO 875 I=1,NP
  875 EMF(I) = EZERO(1) - SLOPE(1) * EMF(I)
C
  880 DO 881 I=1,NP
```

```
      A = (EZERO(1) - EMF(I)) / SLOPE(1)
      IF(A.GT.-1.0.AND.A.LT.15.0)  GO TO 881
      WRITE(OUT,30880)  I, TITRE(I), EMF(I), A
      ERROR = ERROR + 1
      IF(I.EQ.1)  GO TO 888
      K = K + 1
      IF(K.GT.3)  GO TO 1090
  881 CONTINUE
      A = EMF(2) - EMF(1)
      B = EMF(1)
      C = 0.00
      D = 0.00
      NEWERR = 0
      K = 0
      DO 886 I=2,NP
      IF(TITRE(I).LT.TITRE(I-1))  GO TO 882
      B = EMF(I) - B
      IF(A*B.GT.0.00000)  GO TO 884
      IF(I.NE.NEWERR+1.OR.ABS(C).GT.20.0.OR.ABS(D)
                  .GT.20.0)  GO TO 882
      WRITE(OUT,30881)
      GO TO 883
  882 IF(K.GE.2)  ERROR = ERROR + K
      K = 0
      ERROR = ERROR + 1
      IF(ERROR.GT.20)  GO TO 1090
  883 WRITE(OUT,30882)  I, TITRE(I), EMF(I)
      IF(ABS(D).GT.20.0)  WRITE(OUT,30883)
      C = B
      NEWERR = I
      K = K + 1
      IF(K.EQ.2)  ERROR = ERROR - 1
      IF(K.LT.5.AND.I.NE.NP)  GO TO 886
      IF(K.LT.4)  GO TO 884
      WRITE(OUT,30881)
      NEWERR = 0
      WRITE(OUT,30884)
      A = -A
      IF(I.GT.K+2)  GO TO 885
      WRITE(OUT,30885)
      GO TO 1090
  884 IF(K.GE.2)  ERROR = ERROR + K
      IF(ERROR.GT.20)  GO TO 1090
  885 K = 0
      D = C
      C = 0.00
  886 B = EMF(I)
      DO 887 I=2,NC
      IF(CB(1,I).GT.0.000)  GO TO 889
  887 CONTINUE
      A = (EZERO(1) - EMF(1)) / SLOPE(1)
      A = (EZERO(1) - EMF(2)) / SLOPE(1) - A
      IF(A*CB(1,1).LT.0.00)  GO TO 889
      WRITE(OUT,30887)
      ERROR = ERROR + 1
```

```
      GO TO 889
  888 WRITE(OUT,30888)
  889 IF(NFLAG.NE.2)  GO TO 1090
C
C
C     SECTION NINE:  INPUT TERMINATION.
C
C
  900 SEXHUN = 9
      WRITE(OUT,30101)  SEXHUN
      WRITE(OUT,30901)
      GO TO 1090
C
C
C
C     SECTION TEN:   ERROR DIAGNOSTIC PROCEDURES.
C
C
 1000 SEXHUN = 10
      WRITE(OUT,31001)
      IF(NNN.EQ.1)  WRITE(OUT,31002)
      ERROR = ERROR + 1
 1002 NFLAG = 2
      GO TO 1091
C
 1010 IF(KEY1.EQ.LIT(1))  GO TO 1011
      WRITE(OUT,31010)  KEY1, KEY2
      GO TO 1050
 1011 WRITE(OUT,31011)
C
C     ATTEMPT TO RE-ESTABLISH THE DATA SEQUENCE.
C
 1050 NEWERR = ERROR
      IF(NNN.LE.2.AND.ERROR.GE.1.AND.(KEY1.EQ.LIT(12)
                  .OR.KEY1.EQ.LIT(13)
     1))  GO TO 505
 1051 ERROR = ERROR + 1
      WRITE(OUT,31051)  NNN
      IF(NNN.EQ.1)  GO TO 400
      IF(ERROR.GT.20)  GO TO 1090
      IF(KEY1.NE.LIT(1))  GO TO 1060
 1055 NNN = NNN + 1
      READ(IN,10301,END=1002,ERR=1050)  KEY1, KEY2, IVAL,
                  JVAL, VALUE
      WRITE(OUT,31052)
 1060 DO 1061 I=2,29
      IF(KEY1.EQ.LIT(I))  GO TO 1070
 1061 CONTINUE
      IF(KEY1.EQ.LIT(30))  GO TO 1093
      NEWERR = NEWERR + 1
      IF(ERROR.EQ.NEWERR)  GO TO 1055
      WRITE(OUT,31010)  KEY1, KEY2
      KEY1 = LIT(1)
      GO TO 1051
C
```

```
C
 1070 IF(SEXHUN.GT.10)  GO TO 1050
      WRITE(OUT,30001)
      GO TO (1071,1071,1071,1072,1073,1074,1075,1076,1077,
                  1077), SEXHUN
 1071 KEY1 = LIT(1)
 1072 IF(I.LE.10)  GO TO 400
 1073 IF(I.EQ.11)  GO TO 500
 1074 IF(I.LE.13)  GO TO 505
 1075 IF(I.LE.16)  GO TO 605
 1076 IF(I.EQ.17)  GO TO 710
 1077 IF(I.LE.19)  GO TO 800
      IF(I-21)  640, 620, 1078
 1078 IF(I.LE.25)  GO TO 618
      IF(I.LE.28)  GO TO 718
      WRITE(OUT,31077)  NNN
      NEWERR = NEWERR + 1
      GO TO 1055
C
C     END OF INPUT.   OUTPUT ERROR COUNT.
C
 1090 SEXHUN = 10
 1091 WRITE(OUT,30101)  SEXHUN
      IF(NFLAG.EQ.1)  WRITE(OUT,31091)
      IF(NFLAG.NE.1)  WRITE(OUT,31092)
      WRITE(OUT,31093)  ERROR
      IF(ERROR.EQ.0.AND.PROGRM.LT.10)  GO TO 1095
      IF(ERROR.GT.20)  GO TO 1093
      IF(NFLAG.NE.2)  GO TO 600
      IF(ERROR.GT.5)  WRITE(OUT,31099)
      GO TO 1093
C
 1092 WRITE(OUT,31097)  MODE
 1093 PROGRM = 10
      IF(NTITR.GT.1)  GO TO 9999
      WRITE(OUT,30000)
      CALL EXIT(10)
      STOP
C
 1095 GO TO(1100,1200,1300,1400,1500,1600,1700,1800,1900),
                  PROGRM
C
C
C
C     SECTION ELEVEN:   MINIQUAD(74)
C
C
 1100 SEXHUN = 11
      GO TO 1201
C
C
C
C     SECTION TWELVE:   MINIQUAD(75)
C
C
```

```
 1200 SEXHUN = 12
 1201 WRITE(OUT,30101)  SEXHUN
      IF(NC.EQ.1)  MODE = 10
      IF(PKW.LT.10.00)  MODE = 48
      IF(NREF.LT.1.AND.JPROG.NE.1)  MODE = 50
      IF(MODE.NE.2)  GO TO 304
      WRITE(OUT,30002)
      DO 1202 I=1,2
      IF(NDPV(I).EQ.-1)  NDPV(I) = 0
      IF(NDPB(I).EQ.-1)  NDPB(I) = 0
 1202 CONTINUE
      IF(NTITR.GT.1)  GO TO 1220
      I = 25
      J = NC - 1
      K = 1
      M = 2
      IF(JPROG.EQ.1)  I = 0
      IF(JPROG.EQ.2)  K = 0
      L = K
      IF(JPROG.EQ.3)  L = 0
      IF(JPROG.EQ.4)  M = NC + 1
      NEWERR = IPROG
      IF(NEWERR.LE.0)  NEWERR = 1
      IF(NEWERR.EQ.1)  I = I * 2
      IF(JPROG.LT.5)  GO TO 1208
      I = 5
      DO 1206 N=5,JPROG
 1206 I = I * 2
 1208 WRITE(OUTF,20000) TITLE
      NEWERR = 1
      WRITE(OUTF,21201)  NEWERR, NS, NREF, I, K, NC, J,
                         (L, N=1,M)
C
      DO 1210 I=1,NS
      J = IFIX(BETA(1,I))
      A = 10.000 ** (BETA(1,I) - FLOAT(J))
 1210 WRITE(OUTF,21211)  A, J, (JQR(L,I), L=2,NC),
                         JQR(1,I), REFINE(I)
C
 1220 A = SLOPE(1)
      IF(A.LT.0.0000)  A = -A
      A = A / 0.1984161 - 273.15
      CV(1,1) = CV(1,1) + CV(1,2) * NDPV(1) + CV(1,4) *
                NDPV(2)
      WRITE(OUTF,21221)  VZERO(1), A, (CV(1,I), I=2,NC),
                         CV(1,1)
      J = 1
      WRITE(OUTF,21222)  J, EZERO(1), SLOPE(1)
      IF(PROGRM.EQ.2)  WRITE(OUTF,21222)  J
      CB(1,1) = CB(1,1) + CB(1,2) * NDPB(1) + CB(1,4) *
                NDPB(2)
      WRITE(OUTF,21221)  (CB(1,I), I=2,NC), CB(1,1)
C
      IF(NPTOT*(NC-1).LE.1200)  GO TO 1230
      MODE = 70
```

```
      GO TO 304
 1230 NP = NP - 1
      J = 0
      DO 1235 I=1,NP
 1235 WRITE(OUTF,21222)  J, TITRE(I), EMF(I)
      NP = NP + 1
      J = 1
      IF(NFLAG.EQ.2)  J = -1
      WRITE(OUTF,21222)  J, TITRE(NP), EMF(NP)
      IF(NFLAG.EQ.1)  GO TO 600
      IF(PROGRM.EQ.2)  GO TO 1239
      J = 2
      IF(JPROG.EQ.3)  J = 1
      IF(JPROG.EQ.4)  J = 4
      M = NC - 1
      WRITE(OUTF,21201)  J, M, (K, K=1,M)
 1239 J = -1
      IF(PROGRM.EQ.1)  WRITE(OUTF,21222)  J
      IF(PROGRM.EQ.2)  WRITE(OUTF,21311)  J
      GO TO 9999
C
C
C
C     SECTION THIRTEEN:   SCOGS
C
C
 1300 SEXHUN = 13
      WRITE(OUT,30101)  SEXHUN
      IF(NC.EQ.1)  MODE = 10
      IF(IKW.EQ.0)  MODE = 48
      IF(NREF.LT.1.AND.JPROG.NE.1)  MODE = 50
      CALL COPNDP(NDPSTR,MODE,NDPV,NDPB)
      IF(MODE.NE.2)  GO TO 304
      WRITE(OUT,30002)
 1301 IF(NTITR.GT.1)  GO TO 1340
      I = 1
      WRITE(OUTF,21311)  I
      WRITE(OUTF,21311)  I
 1305 I = 19
      WRITE(OUTF,21311)  I
      I = 2
      J = 1
      K = NS - 1
      IF(NC-3)  1311, 1312, 1313
 1311 J = 0
 1312 I = 1
 1313 WRITE(OUTF,21311)  I, J, K
C
      A = 0.000
      B = 1.000
      K = 0
      DO 1322 I=1,NS
      J = -JQR(1,I)
      IF(IKW.EQ.I)  GO TO 1321
```

```
      WRITE(OUTF,21321)  JQR(2,I), JQR(4,I), JQR(3,I), K,
                         J, BETA(1,I)
      GO TO 1322
 1321 A = BETA(1,I)
      M = I
 1322 CONTINUE
      IF(A.LT.-10.00.AND.REFINE(M).EQ.0)  GO TO 1325
      MODE = 48
      IF(REFINE(M).EQ.0)  MODE = 25
      ENDFILE OUTF
      WRITE(OUT,30001)
      GO TO 304
 1325 WRITE(OUTF,21325)  A, B
      I = 5
      IF(JPROG.NE.0)  I = JPROG - 1
      WRITE(OUTF,21311)  I, NREF
      A = 0.0005
      DO 1331 I=1,NS
      IF(REFINE(I).EQ.0)  GO TO 1331
      J = I
      IF(I.GT.M)  J = I - 1
      WRITE(OUTF,21331)  J, A
 1331 CONTINUE
      WRITE(OUTF,21311)  NDPV(1), NDPV(2)
 1340 WRITE(OUTF,21340)  TITLE, NTITR
      A = 0.000
      WRITE(OUTF,21342)  CV(1,3), A, CV(1,2), CV(1,4),
                         CV(1,1),
     1    VZERO(1)
      WRITE(OUTF,21342)  CB(1,3), A, CB(1,2), CB(1,4),
                         CB(1,1),
     1    EZERO(1), SLOPE(1)
      J = 0
      K = NP - 1
      DO 1351 I=1,K
 1351 WRITE(OUTF,21351)  TITRE(I), EMF(I), J
      J = 1
      IF(NFLAG.EQ.2)  J = 9
      WRITE(OUTF,21351)  TITRE(NP), EMF(NP), J
      IF(NFLAG.EQ.1)  GO TO 600
      GO TO 9999
C
C
C
C     SECTION FOURTEEN:  COMICS
C
C
 1400 SEXHUN = 14
      WRITE(OUT,30101)  SEXHUN
      IF(NC.EQ.1)  MODE = 10
      IF(PKW.LT.10.00)  MODE = 48
      IF(NS.LT.1)  MODE = 55
      IF(MODE.NE.3)  GO TO 304
      DO 1405 I=2,NC
      J = I + 21
```

```
      IF(REFINE(J).NE.1)  MODE = 90
 1405 CONTINUE
      IF(MODE.NE.3)  GO TO 304
      WRITE(OUT,30002)
      I = 1
      WRITE(OUTF,21411)  I
      WRITE(OUTF,20000)  TITLE
      I = 2
      J = 1
      IF(NC-3)  1411, 1412, 1413
 1411 J = 0
 1412 I = 1
 1413 WRITE(OUTF,21412)  I, J, NS
C
      DO 1421 I=1,18
 1421 TITLE(I) = 0
      K = 0
      DO 1425 I=1,NS
      TITLE(8) = JQR(3,I)
      TITLE(18) = -JQR(1,I)
 1425 WRITE(OUTF,21411)  JQR(2,I), JQR(4,I), K, TITLE,
                  BETA(1,I)
C
      WRITE(OUTF,21430)  CONC(2), CONC(4)
      WRITE(OUTF,21430)  CONC(3)
      K = 0
      IF(NP.GT.1)  GO TO 1451
      IF(NP.LT.1)  EMF(1) = -ALOG10(CONC(1))
      NP = 1
      GO TO 1455
 1451 J = NP - 1
      DO 1452 I=1,J
 1452 WRITE(OUTF,21452)  EMF(I), K
 1455 K = 1
      WRITE(OUTF,21452)  EMF(NP), K
      GO TO 9999
C
C
C
C     SECTION FIFTEEN:   PSEUDOPLOT
C
C
 1500 SEXHUN = 15
      WRITE(OUT,30101)  SEXHUN
      IF(NC.EQ.1)  MODE = 10
      IF(NTITR.GT.0.AND.NC.EQ.4)  MODE = 40
      IF(NC.LE.1)  MODE = 45
      IF(IKW.EQ.0.OR.PKW.LT.10.00)  MODE = 48
      IF(NS.LT.2)  MODE = 55
      CALL COPNDP(NDPSTR,MODE,NDPV,NDPB)
      IF(MODE.EQ.1.OR.MODE.GT.3)  GO TO 304
      WRITE(OUT,30002)
      IF(NTITR.GT.1)  GO TO 1580
      I = 0
      J = 1
```

```
      WRITE(OUTF,21511)  I, I
      WRITE(OUTF,20000)  TITLE
C
C     GRAPH OPTION
C
      IF(IGRAPH.EQ.0)  GO TO 1520
      K = 3
      IF(NC.EQ.3)  K = 2
      WRITE(OUTF,21511)  I, I, J, K
      K = 1
      IF(JPROG.EQ.1)  K = NTITR
      M = NC + 2
      L = M + 1
      WRITE(OUTF,21511)  K, L, M, I, I
      WRITE(OUTF,21221)  GRAPH
C
 1520 WRITE(OUTF,21511)  NC, NS
      IF(NC.EQ.4)  J = 2
      IF(NC.GE.3)  I = 1
      K = 1
      L = 0
      WRITE(OUTF,21511)  J, I, K, NDPV(1), L, NDPV(2)
C
      WRITE(OUTF,21531)  BETA(1,IKW)
      A = -1.000
      B = 0.000
      WRITE(OUTF,21534)  A, B, B, B
      DO 1535 I=1,NS
      IF(I.EQ.IKW)  GO TO 1535
 1531 WRITE(OUTF,21531)  BETA(1,I)
      C = 0.000
      D = 0.000
      IF(NC-3)  1534, 1533, 1532
 1532 C = FLOAT(JQR(4,I))
      D = FLOAT(JQR(3,I))
      GO TO 1534
 1533 C = FLOAT(JQR(3,I))
 1534 A = FLOAT(JQR(1,I))
      B = FLOAT(JQR(2,I))
      WRITE(OUTF,21534)  A, B, C, D
 1535 CONTINUE
      I = 0
      WRITE(OUTF,21511)  I
      IF(NTITR.GT.0)  GO TO 1570
C
C     OUTPUT FOR SIMULATIONS.
C
      I = NS + NC
      WRITE(OUTF,21511)  I
      K = 1
      DO 1541 I=1,NS
 1541 WRITE(OUTF,21511)  I, K
      K = 2
      J = -1
      WRITE(OUTF,21511)  J, K
```

```
      K = 1
      DO 1542 I=2,NC
      J = J - 1
 1542 WRITE(OUTF,21511)  J, K
 1543 I = NP
      IF(I.EQ.0) I = 1
      A = 0.40
      WRITE(OUTF,21542)  I, A
C
C     GENERATE AND STORE THE HUR NUMBERS FOR EACH
                  COMPONENT.
C
 1544 M = 1
      DO 1548 I=1,NC
      J = I + 21
      K = REFINE(J)
      IF(K.NE.2)  GO TO 1545
      K = 5
      CONC(I) = ALOG10(CONC(I))
 1545 IF(IABS(ISCN).NE.I.OR.NP.GT.0.OR.(ISCN.LT.0.AND.K.
                  EQ.1).OR.
     1   (ISCN.GT.0.AND.K.EQ.5))  GO TO 1548
C
C     COMPONENT SATISFIES SCAN CONDITIONS.
C
      K = K + 1
      IF(K.NE.6)  GO TO 1546
      SCNINC = ALOG10(SCNINC)
      SCNMAX = ALOG10(SCNMAX)
 1546 A = (SCNMAX - CONC(I)) / SCNINC
      M = IFIX(A * 1.0001)
 1548 REFINE(J) = K
C
 1550 WRITE(OUTF,21550)  M
      A = -4.00
      B = 1.00E-6
      DO 1555 I=1,NC
      L = I
 1551 J = L + 21
      WRITE(OUTF,21511)  REFINE(J)
      IF(REFINE(J).GE.5)  GO TO 1553
 1552 WRITE(OUTF,21552)  A, B
      WRITE(OUTF,21552)  CONC(L), SCNINC
      GO TO 1554
 1553 WRITE(OUTF,21552)  CONC(L)
      IF(REFINE(J).EQ.6)  WRITE(OUTF,21552)  SCNINC
 1554 IF(L.NE.2.OR.NC.NE.4)  GO TO 1555
      L = 4
      GO TO 1551
 1555 CONTINUE
      NP = NP - 1
      IF(NP.LT.1)  GO TO 1559
      DO 1557 I=1,NP
      J = I + 1
 1557 EMF(I) = EMF(J)
```

```
      A = - EMF(1)
      CONC(ISCN) = 10.000 ** A
      GO TO 1550
 1559 I = -1
      WRITE(OUTF,21511)  I, I
      GO TO 1599
C
C     OUTPUT FOR TITRATION PSEUDOPLOTS
C
 1570 I = NS + NC*2 + 3
      WRITE(OUTF,21511)  I
      J = 0
      K = 0
      WRITE(OUTF,21511)  J, K
      K = 2
      DO 1571 I=1,NC
      J = -I
 1571 WRITE(OUTF,21511)  J, K
      K = 10
      J = -1
      IF(NC.EQ.3)  J = -2
      WRITE(OUTF,21511)  J, K
      K = 11
      WRITE(OUTF,21511)  J, K
      K = 4
      DO 1572 I=1,NC
      J = -I
 1572 WRITE(OUTF,21511)  J, K
      K = 1
      DO 1573 I=1,NS
 1573 WRITE(OUTF,21511)  I, K
      A = 0.40
      I = 19
      WRITE(OUTF,21542)  I, A
 1580 WRITE(OUTF,21580)  NP
      A = -4.00
      B = 1.00E-6
      J = 4
      CV(1,1) = CV(1,1) + CV(1,2) * NDPV(1)
      CB(1,1) = CB(1,1) + CB(1,2) * NDPB(1)
      DO 1581 I=1,NC
      WRITE(OUTF,21511)  J
      WRITE(OUTF,21552)  A, B
 1581 WRITE(OUTF,21552)  CV(1,I), CB(1,I)
      WRITE(OUTF,21552)  VZERO(1)
      WRITE(OUTF,21534)  (TITRE(I), I=1,NP)
      IF(NFLAG.EQ.1)  GO TO 600
      I = -1
      WRITE(OUTF,21511)  I, I
 1599 CONTINUE
      GO TO 9999
C
C
C
C     SECTION SIXTEEN:   ZPLOT
```

```
C
C
 1600 SEXHUN = 16
      WRITE(OUT,30101)  SEXHUN
      IF(NC.GT.3.OR.NC.EQ.1)  MODE = 10
      IF(IKW.EQ.0)  MODE = 48
      CALL COPNDP(NDPSTR,MODE,NDPV,NDPB)
      IF(MODE.NE.2)  GO TO 304
      WRITE(OUT,30002)
      IF(NTITR.GT.1)  GO TO 1340
      WRITE(OUTF,21311)   IGRAPH
C
C     GRAPH OPTIONS HERE.
C
      A = 0.000
      WRITE(OUTF,21221)  A, GRAPH
      GO TO 1305
C
C
C
C     SECTION SEVENTEEN:   NEW PROGRAM
C
C
 1700 SEXHUN = 17
      WRITE(OUT,30101)  SEXHUN
      IF(MODE.NE.2)  GO TO 304
      WRITE(OUT,30002)
      GO TO 9999
C
C
C
C     SECTION EIGHTEEN:   ECCLES
C
C
 1800 SEXHUN = 18
      WRITE(OUT,30101)  SEXHUN
      IF(NC.EQ.1)  MODE = 10
      IF(IKW.EQ.0)  MODE = 48
      IF(NS.LT.1)  MODE = 55
      IF(REFINE(22)+REFINE(23).GT.6)  MODE = 81
      IF(MODE.NE.3)  GO TO 304
      WRITE(OUT,30002)
      WRITE(OUTF,20000)  TITLE
      WRITE(OUTF,21802)
      WRITE(OUTF,21803)
C
      LIT(22) = LIT(31)
      JVAL = 0
      IF(ISCN)  1801, 1809, 1805
 1801 JVAL = 1
      ISCN = -ISCN
 1805 ISCN = ISCN + 21
      WRITE(OUTF,21805)  LIT(ISCN), SCNINC, SCNMAX
      IF(JVAL.EQ.1)  WRITE(OUTF,21806)
      GO TO 1810
```

```
 1809 WRITE(OUTF,21803)
C
 1810 WRITE(OUTF,21810)
      JVAL = 0
      J = 21
      DO 1815 I=1,NC
      J = J + 1
      IF(REFINE(J).EQ.1)  WRITE(OUTF,21812)
                  LIT(J), CONC(I)
      IF(REFINE(J).EQ.2)  JVAL = 1
 1815 CONTINUE
      WRITE(OUTF,21815)
C
      IF(JVAL.EQ.0)  GO TO 1830
      WRITE(OUTF,21820)
      J = 21
      DO 1821 I=1,NC
      J = J + 1
      IF(REFINE(J).EQ.2)  WRITE(OUTF,21812)
                  LIT(J), CONC(I)
 1821 CONTINUE
      DO 1823 I=1,NS
      IF(JQR(1,I).LT.0.AND.I.NE.IKW)  GO TO 1825
 1823 CONTINUE
      GO TO 1828
 1825 A = 1.0 / (10.00 ** PKW * CONC(1))
      WRITE(OUTF,21812)  LIT(32), A
 1828 WRITE(OUTF,21815)
C
 1830 WRITE(OUTF,21830)
      DO 1838 I=1,NS
      IF(I.EQ.IKW)  GO TO 1838
 1832 K = 0
      DO 1833 J=2,NC
      IF(JQR(J,I).EQ.0)  GO TO 1833
      K = K + 2
      TITLE(K-1) = LIT(J+21)
      TITLE(K) = JQR(J,I)
 1833 CONTINUE
      IF(JQR(1,I).EQ.0)  GO TO 1835
      K = K + 2
      TITLE(K-1) = LIT(31)
      TITLE(K) = JQR(1,I)
 1835 WRITE(OUTF,21835)  BETA(1,I), (TITLE(J), J=1,K)
 1838 CONTINUE
      GO TO 9999
C
C
C
C     SECTION NINETEEN:  MAGEC
C
C
 1900 SEXHUN = 19
      WRITE(OUT,30101)  SEXHUN
```

```
      IF(JPROG.EQ.0.OR.NTITR.GE.JPROG.OR.NFLAG.NE.1)
                  GO TO 1901
      WRITE(OUT,31901)
      GO TO 600
1901  IF(NC.GT.2)  MODE = 10
      IF(IKW.EQ.0.OR.PKW.LT.10.00)  MODE = 48
      IF(MODE.NE.2)  GO TO 304
      WRITE(OUT,30002)
      WRITE(OUTF,20000)  TITLE
      DO 1905 I=1,3
1905  VALUE(I) = BETA(I,IKW)
      WRITE(OUTF,21901) ALPHA(23),REFINE(IKW),VALUE(1),
                  VALUE(3),VALUE(2)
      N = NS - 1
1911  IF(N.EQ.0)  GO TO 1920
      K = 0
      A = 0.000
      DO 1912 J=1,NS
      IF(JQR(1,J).EQ.N)  K = J
      IF(JQR(1,J).EQ.N-1)  A = BETA(1,J)
1912  CONTINUE
      IF(K.EQ.0)  GO TO 1915
      VALUE(1) = BETA(1,K) - A
      VALUE(2) = 0.000
      VALUE(3) = 0.000
      IF(REFINE(K).NE.2)  GO TO 1913
      VALUE(2) = VALUE(1) - (BETA(1,K) - BETA(2,K))
      VALUE(3) = VALUE(1) + (BETA(3,K) - BETA(1,K))
1913  L = NS - N
      WRITE(OUTF,21901)  NUMBER(L), REFINE(K), VALUE
      N = N - 1
      GO TO 1911
1915  MODE = 58
      ENDFILE OUTF
      GO TO 304
1920  DO 1921 I=21,30
      IF(REFINE(I).LT.0)  REFINE(I) = 0
1921  CONTINUE
      IF(NC.EQ.1)  NDPV(1) = 0
      IF(NDPV(1).EQ.-1)  NDPV(1) = NDPB(1)
      IF(NDPB(1).EQ.-1)  NDPB(1) = NDPV(1)
      WRITE(OUTF,21921)  ALPHA(22), ALPHA(26), ALPHA(15),
                  REFINE(21),
     1  VZERO
      WRITE(OUTF,21921)  ALPHA(8), SIHN(2), ALPHA(22),
                  REFINE(22),
     1  (CV(I,1), I=1,3)
      WRITE(OUTF,21922) ALPHA(22), REFINE(23), NDPV(1),
                  (CV(I,2), I=1,3)
      WRITE(OUTF,21921)  ALPHA(8), SIHN(2), ALPHA(2),
                  REFINE(26),
     1  (CB(I,1), I=1,3)
      WRITE(OUTF,21922)  ALPHA(2), REFINE(27), NDPB(1),
                  (CB(I,2), I=1,3)
```

```
      WRITE(OUTF,21921)  ALPHA(5), ALPHA(26), ALPHA(15),
                         REFINE(29),
     1  EZERO
      WRITE(OUTF,21921)  ALPHA(19), ALPHA(12), ALPHA(16),
                         REFINE(30),
     1  SLOPE
      DO 1931 I=1,NP
 1931 WRITE(OUTF,21351)  TITRE(I), EMF(I)
      A = -1.000
      WRITE(OUTF,21351)  A
      IF(JPROG.EQ.0.AND.NFLAG.EQ.1)  GO TO 600
C
C
C     THE END.
C
C
 9999 ENDFILE OUTF
      WRITE(OUT,30003)  PROGRM
      IF(NPTOT.GT.0)  WRITE(OUT,30004)  NPTOT
      IF(PROGRM.EQ.10)  WRITE(OUT,30005)
      WRITE(OUT,30000)
      IF(KEY1.EQ.LIT(30).AND.IVAL.NE.0)  PROGRM = 11
      CALL EXIT(PROGRM)
      STOP
      END
```

```
C
C
C
      SUBROUTINE FREED(X,Y,N,NFLAG,IN,OUT,DIGIT,LFINIS,
                       LVESSL,SPECIE)
C
C
C
      DOUBLE PRECISION  X, Y
      INTEGER CARD(80), NUMBER(10), DIGIT(10), LIT(5),
                       OUT, SPECIE(5)
      LOGICAL NEG, FILLY
      DATA LIT/' ',',','.','-','+'/
C
C
  101 FORMAT(80A1)
  102 FORMAT(' ',10X,I5,10X,2F10.3)
  103 FORMAT(//'0','ERROR DETECTED ON CARD
                       NUMBER',I4,/'0',10X,80A1,///)
  204 FORMAT('0','NEGATIVE VOLUME')
C
C
   10 ISTART = 1
      FILLY = .FALSE.
      READ(IN,101,END=80,ERR=90)  CARD
C
      NUM = 0
      DO 15 I=1,80
      IF((NUM.EQ.2.OR.I.GT.20).AND.CARD(I).NE.LIT(1))
                       GO TO 90
```

```
      IF(CARD(I).EQ.LIT(2))    NUM = NUM + 1
   15 CONTINUE
C
C
   20 NUM = 0
      NPOINT = -1
      NEG = .FALSE.
      DO 25 I=ISTART,20
      IF(CARD(I).EQ.LIT(1))  GO TO 25
      IF(CARD(I).EQ.LIT(2))  GO TO 30
      IF(CARD(I).NE.LIT(3))  GO TO 21
      IF(NPOINT.NE.-1)  GO TO 90
      NPOINT = NUM
      GO TO 25
   21 IF(CARD(I).NE.LIT(4))  GO TO 22
      IF(NEG.OR.NUM.NE.0)  GO TO 90
      NEG = .TRUE.
      GO TO 25
   22 NUM = NUM + 1
      IF(NUM.GT.10)  GO TO 90
      DO 23 J=1,10
      IF(CARD(I).EQ.DIGIT(J))  GO TO 24
   23 CONTINUE
      IF(NUM.NE.1.OR.CARD(I).NE.LIT(5))  GO TO 90
      NUM = 0
      GO TO 25
   24 IF(J.EQ.10)  J = 0
      NUMBER(NUM) = J
   25 CONTINUE
      I = 0
      IF(.NOT.FILLY)  GO TO 90
C
C
   30 Y = 0.000
      IF(NUM.EQ.0)  GO TO 90
      ISTART = I + 1
      IF(NPOINT)  34, 40, 35
   34 NPOINT = NUM
   35 IF(NPOINT.EQ.1)  GO TO 37
      NPM1 = NPOINT - 1
      DO 36 I=1,NPM1
   36 Y = Y + FLOAT(NUMBER(I)) * 10.00 ** (NPOINT - I)
   37 Y = Y + FLOAT(NUMBER(NPOINT))
C
   40 NPOINT = NPOINT + 1
      IF(NPOINT.GT.NUM)  GO TO 50
      DO 45 I=NPOINT,NUM
   45 Y = Y + FLOAT(NUMBER(I)) / 10.00 ** (I - NPOINT + 1)
C
   50 IF(FILLY)  GO TO 60
      FILLY = .TRUE.
      X = Y
      IF(.NOT.NEG)  GO TO 20
      WRITE(OUT,204)
      GO TO 90
```

```
C
   60 IF(NEG)  Y = -Y
      WRITE(OUT,102)  N, X, Y
      RETURN
C
   80 NFLAG = 2
      GO TO 82
   81 NFLAG = 1
   82 X = 0.00
      Y = 0.00
      RETURN
C
   90 IF(CARD(1).EQ.LFINIS)  GO TO 80
      DO 91 I=1,5
   91 SPECIE(I) = CARD(I)
      IF(CARD(1).EQ.LVESSL)  GO TO 81
      NFLAG = NFLAG - 1
      IF(N.EQ.0)  RETURN
      WRITE(OUT,103)  N, CARD
      GO TO 82
      END
      SUBROUTINE COPNDP(NDPSTR,MODE,NDPV,NDPB)
      INTEGER  NDPV(2), NDPB(2)
      IF(NDPV(1).EQ.-1)  NDPV(1) = NDPB(1)
      IF(NDPV(2).EQ.-1)  NDPV(2) = NDPB(2)
      IF(NDPB(1).EQ.-1)  NDPB(1) = NDPV(1)
      IF(NDPB(2).EQ.-1)  NDPB(2) = NDPV(2)
      J = NDPV(1) * 10 + NDPV(2)
      IF(NDPSTR.NE.-1.AND.NDPSTR.NE.J)  MODE = 95
      NDPSTR = J
      J = NDPB(1) * 10 + NDPB(2)
      IF(NDPSTR.EQ.J)  RETURN
      J = 0
      IF(MODE.EQ.95)  J = 1
      MODE = J + 96
      RETURN
      END
```

APPENDIX A6

Review of the biological significance of the low-molecular-weight Iron complexes

The following review has been published in "Metal Ions in Biological Systems", Vol.7 (H.Sigel, ed.) Marcel Dekker, New York, 1978. It was researched and initially written by the author as part of the present work.

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BIOLOGICAL SIGNIFICANCE OF LOW MOLECULAR WEIGHT IRON(III) COMPLEXES

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1. INTRODUCTION

In the past a great deal of controversy, often verging on confusion, has surrounded the topic of low molecular weight (low mol wt) complexes in physiclogical environments. Much of the disagreement may be attributed to the interdisciplinary nature of the subject; differing viewpoints, multifarious experimental approaches, and even partisan logic play a part. It is therefore prudent to commence by attempting to place these low mol wt bioinorganic species in their true perspective.

Owing to the very powerful metal-binding properties of the macromolecules which occur in biological fluids, the concentrations of metal ions available for the formation of low mol wt complexes are severely limited. Hence the concentrations of these low mol wt complexes are extremely small, usually immeasurably so. Nevertheless, these concentrations are orders of magnitude greater than the corresponding aqueous metal ions. Moreover, in spite of their low concentrations, the low mol wt complexes are believed to be of immense biological significance.

Probably the most important function of these low mol wt complexes involves the transport of metal ions (1) through biological membranes and (2) both into and out of macromolecules/membrane-bound biological receptor sites [1]. Metal ions held in the low mol wt fraction are far more readily accessible than those bound within proteins. Macromolecules cannot passively diffuse through membranes. Hence, in both the above-mentioned attributes small complexes possess a kinetic advantage over their macromolecular counterparts.

Additional features of low mol wt complexes include (1) an ability to alter the potential of certain redox couples, (2) their role in the process of dissolving certain metal prosthetics, and (3) the selectivity that low mol wt complexes confer upon metal ions in vivo. In this last respect it has been suggested that differences in the distribution of low mol wt complexes might be exploited during metalloprotein synthesis, whereby differences between the stereochemistries of related complexes are recognized [2].

LOW MOLECULAR WEIGHT IRON(III) COMPLEXES

Turning more specifically to Fe(III) complexes, their physiology is governed to a large extent by the chemical properties of low mol wt species. In particular the concept of bioavailability is dominant. This emanates out of the pronounced tendency of this metal ion to form insoluble hydrolysis and polymerization products and leads to an ambivalent importance of the Fe(III) low mol wt complexes.

On the one hand, chelates can enhance bioavailability because, through suppressing irreversible polymerization, the metal ion is held in solution. On the other hand, evolution has circumnavigated this problem of insolubility by producing unique mechanisms which lessen the need for these low mol wt mediators.

In some microorganisms, the evolution of improved means for assimilating and handling iron has produced some hydroxamate and phenolate ligands with very tenacious Fe(III) binding capacities. These siderochromes are of great biological importance and are discussed in Chap. 3. Much research has been directed at elucidating the nature and properties of the iron complexes involved in plant physiology, but this topic cannot be treated in detail here. This chapter deals with the biological significance of low mol wt complexes of Fe(III) in the context of mammalian physiology. The objectives are threefold: (1) to review work which directly implicates low mol wt complexes in iron metabolism, (2) to present the results of computer simulation studies which reveal the nature and concentration of these low mol wt complexes occurring under in vivo conditions, and (3) to consider the mechanisms through which low mol wt complexes participate in the regulation of iron metabolism.

Any review dealing with the role of low mol wt complexes and iron metabolism must reflect the early and major contribution to this subject made by the researches of Saltman and co-workers [3-23]. The basic ideas behind their pioneering approach have been, and continue to be, substantiated by other investigators. It is now clear that, whether low mol wt compounds do in fact mediate in a specific biological process, experiments must be designed with this possibility in mind. Neglect of equilibrium considerations in general and of chelation phenomena in particular have been responsible for many conflicting

reports not only as discussed for iron but also for other transition metals.

2. AQUEOUS COORDINATION CHEMISTRY

The aqueous coordination chemistry of Fe(III) has often been reviewed. A number of articles have specifically dealt with the biochemical implications of its chemical versatility [19, 22, 24]. No doubt these properties account for the wide-ranging physiological utilization of this metal reflected, for example, throughout this volume.

Both Fe(II) and Fe(III) form complexes with a broad selection of ligands, showing a marked preference for octahedral coordination. With a few exceptions, chelation is much stronger with the higher charged, smaller, Fe(III) ion. Fe(III) binds substances such as polyols, organic oxyacids, and phosphates very powerfully and may be classified in HSAB terminology as hard, intermediate.

Many of the biological functions of iron hinge upon its ability to act as a redox catalyst. Facile reversibility is an essential feature of the Fe(II)-Fe(III) couple which makes it ideal as a physiological electron sink and source. With a standard electrode potential of some 0.77 V (25°C), Fe(II) ion reacts with molecular oxygen even at reasonably high pH values. Although many agents, including a number of metabolites, are able to reduce Fe(III) ions, the pronounced advantages the latter enjoy in all aerobic environments needs to be stressed. Only ligands which form high-spin complexes, thereby increasing the electrode potential, stabilize Fe(II) over Fe(III). Examples are porphyrin-like molecules, o-phenanthroline and bipyridyl, the first named being extremely potent in this respect. Most other ligands lower the electrode potential and thus enhance the stability of the Fe(III) state. This should be borne in mind whenever reduction is postulated to occur in vivo. Such reactions probably only occur spontaneously in the presence of high local concentrations of reducing metabolite or under the influence of special enzyme mechanisms.

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The three most conspicuous chemical properties of aqueous Fe(III) ions are closely related, and so it is easier to outline them together. They are (1) the marked tendency to hydrolyze, (2) the extreme insolubility of the hydrolysis products, and (3) the common and readily occurring hydrolytic polymerization reactions. Many of the reported contradictions concerning iron metabolism stem from an inadequate appreciation of these properties. Even when Fe(III) appears to be solubilized it may well be present in large aggregates [19] which are unable to pass through biological membranes and tend to be adsorbed onto glass or other surfaces.

Extensive studies of Fe(III) hydrolysis have been made, for example, by Hedström [25] and Biedermann [26]. They report the formation of $Fe(OH)^{2+}$, $Fe(OH)_2^{+}$, $Fe_2(OH)_2^{4+}$, as well as higher polymers. The dinuclear dimer and the high polymers involve hydroxo bridging. With increasing alkalinity, more highly condensed species are produced leading to the formation of the classical ferric-hydroxy gel.

As the solubility product of $Fe(OH)_3$ is below 10^{-36} mol^4 dm^{-12}, it is clear that in order for Fe(III) to be maintained in solution at neutral or higher pH, protection by strong binding to suitable ligands is necessary. Polymerization is then restricted by the reduction in the number of aquated sites vulnerable to hydrolysis, the lowering of the effective charge on the metal ion, and the consequent diminished acidity of any coordinated water molecules remaining. It is important to note that although the possibility of dinuclear/oligonuclear Fe(III) complex formation in experimental solutions should never be neglected, the exceedingly low concentrations of free Fe(III) ion, combined with the preponderance of ligands in vivo, makes their existence in biofluids much less likely. When ligands are not strongly bound to Fe(III) or if they occupy only two or three of the coordination sites on the metal, polymerization reactions do occur. For example, both citrate [12] and fructose [21] form high mol wt structures. Nevertheless, when in excess, the ligands can suppress polymer formation [13, 19, 21].

Spiro and Saltman have drawn attention to a number of interesting similarities exhibited by these high mol wt Fe(III) polymers [19]. They do not appear to be just metastable intermediates in the precipitation process as they cannot easily be encouraged to proceed further along such a route. They form compact, well-separated spherical particles around which a coating of the ligand tends to form and thus prevent further hydrolysis. Certain analogies can be drawn with the ferric polyhydroxy-phosphate core of the iron storage agent, ferritin. This subject will be pursued further in Sec. 3.2.2.

Due to the experimental difficulties which we have just described, relatively little work has been published about Fe(III) complexation equilibria in aqueous solution especially at physiological pH values. Citrate is a ligand of obvious potential biological importance. Nevertheless there is little known about the Fe(III) complexes it forms except when the pH is low. A number of suggestions have been put forward on the grounds of potentiometric studies; while the species Fe·citrate·OH^- (i.e., with the ligand being $citrate^{3-}$) has been postulated most often, [12, 13, 27-30] whether it competes successfully with Fe·citrate·$(OH)_2^{2-}$ [28], Fe_2·$citrate_2$·$(OH)_2^{2-}$ [29], or Fe·$citrate_2$·$(OH)_2^{5-}$ [13] is still unclear. Another unresolved question concerns the participation of the citrate hydroxyl function in the binding. The marked tendency for Fe(III) ion to form ligand-hydroxy complexes argues against its formation. Although Fe(III) ion complexation by carbohydrates and polyols is well known and has been the subject of several researches [12, 31], exceedingly little quantitative information is available. Davis and Deller have assessed the chelating ability of a series of sugars by a solubility technique [32]. Those with hydroxyl groups on carbon atoms 1 and 3, namely fructose, sorbose, and tegatose, were found to bind Fe(III) most strongly.

3. EVIDENCE FOR LOW MOLECULAR WEIGHT COMPLEXES INFLUENCING IRON METABOLISM

The emphasis in this section concerns what are believed broadly to be the major roles of low mol wt Fe(III) complexes, namely in the transport of the metal across membranes and in the exchange of iron between macromolecules.

3.1. Metal Ion Transport Through Membranes

The bioassimilation of substances and their subsequent distribution between body compartments depends primarily upon the ease with which they traverse biological membranes [33]. Consequently, a great deal of research has been concerned with the ways in which transport across membranes is accomplished in vivo.

Broadly speaking, transport processes are classified as either active or passive. Active transport is characterized by at least a majority of the following features: (1) consumption of metabolic energy, (2) inhibition by metabolic poisons, (3) partial or complete substrate specificity, (4) transport against concentration gradients and, if the substance is charged, against a potential gradient, and (5) saturation of the transport mechanism at higher substrate levels. On the other hand, passive transport is diffusion-controlled and only permits the transit of lipophilic molecules. Charged species are unable to penetrate the membrane due to the high energy required to move them from an aqueous into a lipid phase.

In three important instances, the classification of membrane transport processes as either active or passive is inadequate [33].

1. In the intermediate case of facilitated diffusion. This kind of mechanism is saturatable and can move charged

species. It does not however rely upon oxidative metabolism. Neither can it work against a concentration gradient. The motion of the membrane-bound receptor is slight and the procedure is probably accomplished simply by a regularly reversing shift of charge density.

2. When pores exist in the membrane. A prominent example of this is provided by glomerular filtration in the kidneys. Only the passage of molecules larger than albumin (mol wt ≃ 70,000) is restricted.
3. The processes of pinocytosis and phagocytosis whereby larger particles can traverse membranes through the extrusion of vesicles.

The movement of transition metal ions across a biomembrane is complicated by a number of factors. The most obvious of these is the ability to form complexes with both high and low mol wt components. While this propensity can clearly be exploited in facilitated diffusion or active transport mechanisms, it also means that the metal ion can passively diffuse through the lipid membrane in the form of a neutral complex. Interpretation of experimental results is made difficult because every one of the criteria used to identify the kind of transport process can be ambiguous. Protein binding in the cell can lead to a net uptake of the metal ion. This may be as a result of irreversible metalloprotein synthesis as well as the labile protein interactions that should always be expected when investigating the physiology of transition metal ions. Coupled with other phenomena, the synthesis of ferritin in mucosal cells has a pronounced, albeit sometimes indirect, effect upon observations pertaining to iron absorption. Thus transport phenomena can be altered by protein synthesis inhibitors, other metabolic poisons, and the deprivation of metabolic energy. Complexation can quite generally cause passive movement of the metal ion against a concentration gradient. Often this will show the typical effects of saturation. It may well be specific for a certain kind of metal ion or for a group with similar chemical properties. Most deceptively,

changes in species distribution between the media on either side of the membrane, due to differences in composition or pH, will tend to produce a flux for reasons by no means easy to discern. Finally, changes in oxidation state which certain transition elements can undergo introduces another type of complication.

The above-mentioned considerations do not imply that active transport of transition metal ions never occurs; only that the interpretation of experimental evidence is not straightforward.

3.1.1. Lipophilicity

As most semipermeable membranes, including those surrounding cells, are certainly not endowed with active transport mechanisms specific for transition metal ions, passive diffusion constitutes the only means whereby metal ions can penetrate these. Under such circumstances neither protein-bound nor free (i.e., aqueous) metal ions can enter directly and so low mol wt lipophilic complexes become the key to understanding how the metal is distributed between body compartments. Glomerular filtration by the kidneys provides a most important exception, but otherwise these principles, well known for ligands, have also been established for metal complexes [33-35].

In the case of Fe(III), the chemical nature of the element imposes restrictions not applicable to other essential trace elements such as zinc or copper, at least not to the same extent. So there is undoubtedly a greater need for special biological methods to handle this element. Nevertheless, substantial evidence exists that lipophilic Fe(III) complexes traverse biomembranes just like lipophilic complexes of other metals.

This is well illustrated by studies of the transfer of iron from synthetic iron chelates to reticulocytes. Normally these cells take up iron from transferrin in plasma. Iron presented in the form of its EDTA chelate is unavailable for incorporation into hemoglobin [36]. However, if this charged, hydrophilic complex is replaced by others with higher lipid/water partition coefficients, the failure to deliver iron to the cell is reversed. Rubin has used a series

of ligands related to EHPG [34]. He found that the more lipophilic the complex, the greater the amount of iron incorporated into the cell by transfer through the membrane.

Similar results have been found in another investigation [37]. Complexes which are both lipophilic and strongly formed simulate the functions of transferrin in that they inhibit nonselective membrane *adsorption* and promote its utilization within the reticulocyte. Highly polar, anionic iron chelates such as those formed by EDTA and DTPA inhibit both nonselective deposition of iron on the cell wall and the incorporation of the metal into hemoglobin.

The role of lipophilic complexes in iron absorption is a question that arises on several occasions throughout this review. Here it is necessary only to mention a few clearcut examples. The fat-soluble chelate ferrocene is almost quantitatively absorbed following oral administration [38]. As opposed to their poor absorption of normal "inorganic" iron, sla-mice (see Sec. 3.3.1) fed another (unidentified) lipophilic organic iron compound showed effective transfer of the iron from the lumen into plasma [39]. These very strong and/or inert complexes provide good illustrations because they survive the sharp changes of pH experienced as they pass along the gastrointestinal tract and also because they are unscathed by the variety of iron-binding components in the intestinal fluid and within mucosal cells. Generally the absorption of metal ions administered orally is relatively limited and in sharp contrast to the rapid and complete uptake of complexes associated with intraperitoneal, intramuscular, or subcutaneous injection.

Another instructive demonstration is afforded by the transfer of iron through rat gut segments in the presence of 8-hydroxyquinoline or its sulfonic acid analog [40]. Compared with normal controls which absorbed about 8% of the ferric chloride dose, 8-hydroxyquinoline promoted the absorption of about 35%, while 8-hydroxyquinoline-5-sulfonic acid permitted the uptake of less than 1%. These effects are entirely attributable to the charge on the iron complex formed by the respective ligands.

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Fructose is a most effective agent for moving iron(III) across membranes [6]. It strongly promotes oral iron absorption [7, 20, 41]. At high concentrations, iron polymerizes in the presence of fructose, but the polymers also dissociate fairly readily [21]. Definitive information concerning the nature of the low mol wt complex formed between iron and fructose in the presence of the polymer is not yet available. However, a neutral species seems highly probable. The inference made by comparison with the gluconate-iron(III) system [31] supports this.

There is some uncertainty with regard to the effect of EDTA upon iron absorption. The negatively charged complexes it forms are not expected to be well absorbed. There seems little doubt that in vivo EDTA suppresses iron uptake [41-43]. NTA is much more effective at increasing the unidirectional influx of iron into the mucosal cell [44], but this could be due to factors other than the difference in complex lipophilicity.

The series of investigations by Princiotto, Rubin, and coworkers extending over two decades has clearly revealed how the distribution of iron within the body and the route of excretion the metal subsequently takes depend upon the lipophilicity of the ferric chelate [34, 37, 45-51]. In essence, nonpolar compounds are excreted by the liver. This is part of the rationale behind the design of enterohepatic chelating agents [52]. Polar complexes are confined to the extracellular space until they are excreted by the kidneys. Thus, complexes which enhance iron uptake by reticulocytes also induce biliary iron excretion [37, 47]. However, bile duct ligation or changes in the chelate structure which reduce the lipid/water partition coefficient shunt the iron excretion into the urine [47, 48]. Esterification of acid groups on certain chelating agents was also observed to cause a shift in excretion pattern toward the fecal route [53].

3.1.2. Active Versus Passive Transport

Much controversy surrounds the mechanisms whereby iron is normally transported across membranes in vivo, particularly in iron absorption.

Numerous investigators have reported results consistent with a simple process of passive diffusion of iron across the gut wall [9, 11, 54-57]. Some even find that the transfer is unaffected by withdrawal of oxidative metabolic energy [54-56]. Interestingly, Terato et al. [57] showed that the absorption of iron chelates and polymers could be related to their molecular weights as well as to the iron concentration in the mucosal medium.

The physiological importance of the passive movement of iron through membranes is indicated by the uptake of the metal ion by rat liver slices [3, 4]. The fact that iron passes through the cell wall in both directions in a manner not affected by agents which inhibit respiration or the utilization of metabolic energy argues against an active process. Further, conditions which disrupt the integrity of the cell membrane do not change the iron metabolism. The accumulation of the metal ion against a concentration gradient appears to be due to iron binding by specific sites within the cell.

It should be noted, however, that evidence exists for the energy-dependent accumulation of iron by the mitochondria of rat liver [58]. Romslo suggests that the effect of added ATP was twofold: it provided energy and it chelated the iron.

Early research by Hahn et al. [59] convinced them that the transfer of iron across the intestinal epithelium is influenced by cell metabolism. Since then it has been shown repeatedly that a process dependent upon metabolic energy is involved [11, 54, 55, 60-64]. Many of these results may merely reflect a change in cell status with regard to either ferritin synthesis or electrode potential under the experimental conditions.

Some work demonstrating an active transport mechanism for Fe(II) ion has been published [60-63, 65]. Iron is thereby transferred against potential and concentration gradients. The process is inhibited by deprivation of metabolic energy or the addition of metabolic blocking agents [60-63]. The two-step process shows rapid uptake by the cell followed by slow release at the serosal surface [61]. There is some disagreement about the saturation kinetics of

the system [61, 63]. This may be due to the loss of cellular function which can occur when gut sacs are incubated over several hours [56].

A question which merits a great deal of attention concerns the oxidation state of iron during absorption. Belief that only Fe(II) could be carried across the membrane was once very widespread. However, it is now known that early work was aggravated by the extreme insolubility of Fe(III) salts in neutral/alkaline biofluids. Iron is equally well absorbed in either oxidation state provided it is kept in solution [9, 23, 40, 66]. For Fe(III) ion this means it must be complexed. Manis and Schacter have suggested that both Fe(II) and Fe(III) are taken up by the mucosal cell but that active transport to the serosal surface is relatively specific for the divalent cation [61]. Thus the mucosal Fe(II) pool turns over relatively rapidly [65]. A little is converted into Fe(III) which acts as a storage depot for excess. Although reducing agents such as cysteine, liberated in the breakdown of proteins, may cause Fe(II) to be formed in the gastrointestinal tract [40], it seems unlikely that this form persists in intestinal fluid under normal circumstances (vide supra). Saltman has pointed to the drastic conditions required to reduce Fe(III) in vivo [23].

In conclusion, it seems that both active and passive transport mechanisms have a role to play. The relative importance of each is difficult to evaluate. Dowdle et al. have suggested that the active transport mechanism for iron will become important if passive diffusion is restricted [60]. This fits the fact that at low concentrations iron absorption appears to be a saturatable process, but at higher concentrations the process exhibits unlimited capacity [40]. Cyclic life processes obviously require some active physiological perturbation to keep them going, but it is well to remember that the efficiency for which Nature is renowned makes it likely that these processes are used sparingly and selective exploitation of spontaneous reactions and of equilibrium conditions are employed instead whenever possible.

3.1.3. Low Molecular Weight Complexes in Iron Absorption

(a) Bioavailability in the Lumen. Absorption of iron takes place throughout the intestine but mainly in the duodenum. An exceedingly large number of studies have shown that a principal factor involved is simply that of iron solubility (vide infra). Anything which tends to make iron more soluble, thereby preventing hydroxide precipitation as the pH rises upon leaving the acid stomach, will promote iron absorption. Therefore Fe(II) forms are preferred to Fe(III). Furthermore, both chelating agents and reducing agents are almost always beneficial. Alternatively, substances which form insoluble salts depress absorption as do any agents which promote the formation of inert polymers or powerful hydrophilic complexes.

Substances which promote absorption by complexation include (1) amino acids, (2) sugars and polyols, (3) most proteins, probably following proteolysis, and (4) some organic oxyacids [40, 67, 68]. These promoting effects appear to be quite variable; lack of enhancement has been reported [69]. However, fructose and histidine seem most instrumental (Sec. 3.1.1).

It has been suggested that some of these substances promote iron absorption by providing the intestinal epithelium with oxidative metabolic energy. In their studies on active transport, Dowdle et al. found that iron transfer was enhanced if glucose was replaced by fructose in the incubation medium [60]. Manis and Schacter noted that mannose was even better than fructose in this respect [61]. It seems that complexation is in all likelihood a more dominant factor, however, Jacobs et al. could not confirm the effectiveness of mannose [63]. Transfer, they found, was increased by citric acid and α-ketoglutaric acids, but other metabolites gave equivocal results. The minimum concentration of carbohydrate sufficient to prevent iron hydroxide precipitation provides a measure that permits a correlation between iron-chelating and absorption-promoting ability: fructose > sorbitol > glucose > sucrose [7]. Proposals that the enhancing effect of amino acids is achieved by the active transport mechanism in intestinal epithelium which is specific for these metabolites are

refuted by the observation that L isomers do not do significantly better than the D forms [70].

Although Fe(III) and Fe(II) are absorbed equally well if they are both properly solubilized [40], in practice the difficulty in fulfilling this condition places Fe(III) at a distinct disadvantage [68]. Hence, reducing agents promote absorption. Ascorbic acid and cysteine are known to be most effective [68, 71]. The ability of these ligands to form soluble Fe(II) chelates is probably an equally important function [71]. Reduction can, in addition, liberate Fe(III) ions bound in inert metalloproteins [40]. Substances which depress absorption by decreasing transfer into the mucosal cell include phosphates, phytates, carbonates, oxalate, hydroxide, iron-binding proteins, and synthetic chelating agents [40, 67, 68, 71].

Suggestions which assign the control of iron absorption to substances secreted into the intestinal milieu have produced a fair measure of confusion and much controversy. Such secretions have been claimed to regulate the passage of iron through the intestinal membrane by chelating the metal while it is rendered soluble in the acid content of the stomach. Whether this binding enhances or inhibits absorption is, however, in the face of contradictory assertions, unclear, and governance by such means is currently thought unlikely [56, 68, 71]. More attractive is the concept that iron remains soluble by binding to either high mol wt mucopolysaccharides [72] or to low mol wt ligands. These may be from food or from the amino acids secreted into the lumen. In any event, the macromolecular component is unable to penetrate the intestinal epithelium. Hence, iron must be released either to low mol wt chelators for passive diffusion into the cell or to acceptor sites on the intestinal brush borders. The latter process might need to be mediated by small ligands.

Actually, the effects of all these agents upon iron absorption should be seen as the result of an influence on the metal in a multicomponent, multiphase equilibrium system as the solution undergoes a transition from acid to neutral pH. Hydrochloric acid in gastric

juice dissolves sparingly soluble compounds and releases iron in food [68, 73]. Reduction of Fe(III) is routinely accomplished by cysteine and glutathione end products of protein digestion [40]. However, the reversal to the higher oxidation state becomes increasingly favored as the medium gets more alkaline; it is under such circumstances that chelation assumes paramount importance if precipitation (or inert polymer formation) is to be forestalled and the metal is to remain bioavailable.

(b) Transfer into the Mucosal Cell. It is generally accepted that whatever active transport mechanisms may be associated with iron absorption, these are not located at the mucosal surface [40] in spite of a few contrary assertions. This being the case, transfer into the mucosal cell can proceed by either passive or facilitated diffusion (Sec. 3.1). The former process will hinge upon the concentration of lipophilic low mol wt species in the intestinal fluid while the latter would probably not depend on the charge of the complex but rather its concentration, binding strength, and stereochemistry. Little research has been aimed at distinguishing between these two possibilities because of the problems connected with identifying low mol wt complexes formed in biofluids (Sec. 4). However, iron deficiency anemia has been named second only to protein malnutrition in the number of people it affects [74], so the need to find better iron supplementing therapeuticals without the side effects commonly caused by these hematics is obvious. Understanding how an agent facilitates penetration of the mucosal cell will certainly help.

Forth and Rummel have proposed that acceptor sites exist on the brush border of mucosal cells and that these compete for iron with ligands in the lumen [40]. They attribute the different absorptive capacities of gut segments to diminishing stability of the complexes formed with binding sites on segments taken closer to the ileum. They categorize ligands according to binding ability:

1. Strong ligands whose complexes do not release the metal to the brush borders

2. Intermediate ligands which do yield iron for absorption but which are capable of preventing hydrolytic precipitation
3. Weak ligands which do not bind in the face of competition from hydroxide ion

Recently, Sheehan has published a study on the unidirectional uptake of iron which suggests that this occurs by passive diffusion [44]. He found a linear relationship with respect to concentrations between 0.1 and 10 mmol dm^{-3}. This is in good agreement with many of the observations of Saltman. Sheehan also noted that uptake was a process with a low energy of activation. No features characteristic of a rate-controlling step could be demonstrated. Unidirectional uptake depended only on the iron concentration and the nature of the complex: Fe-ascorbate > Fe-NTA >> Fe-EDTA.

Two important instances where penetration into the mucosal cell is accomplished with ease should be mentioned:

1. Heme absorption: Following the degradation of hemoglobin in food, the heme moiety is drawn into the mucosal cell intact [75]. In fact, it is the only low mol wt iron chelate experimentally demonstrated within mucosal cells under physiological conditions [40], no doubt reflecting the inert nature of this complex. Absorption of hemoglobin iron is not affected by those factors so important in determining the availability of "inorganic" iron in intestinal fluid, e.g., ascorbic acid, phytates, and DFO [67, 71]. It has been shown that differences in iron absorption of heme can be related to molecular weight, monomers being absorbed best [71]. Polymer formation of porphyrins is probably the reason they are not absorbed as well as hemoglobin iron [40]. Once the heme has entered the mucosal cell, it is split enzymatically (possibly by xanthine oxidase) and thereafter joins the same pathway as that taken by absorbed inorganic iron [75].

2. Bantu siderosis: This pathology, widespread in southern Africa, has been directly attributed to the formation of iron-carbohydrate complexes which remain soluble and undissociated in intestinal fluid and which pass easily through the epithelium into

plasma [23]. The consequences of this uncontrolled bypass of normal iron absorption pathways is excessive deposition of the metal in the liver and spleen which ultimately proves fatal. The complexes are apparently formed with ligands generaged during fermentation: the Bantu peoples consume large quantities of maize-based "beer" brewed in iron pots and a similar iron overload is observed in sweet-wine alcoholics [23]. It is noteworthy that even when ferrous sulfate is ingested with maize, alcohol, or unfermented gruel, iron absorption does not become exceptionally high [76]. Neither does the consumption of teff, an Ethiopian cereal grass having a sizable iron content, cause a comparable siderosis [76].

3.2. Macromolecules and Low Molecular Weight Fe(III) Complexes

3.2.1. Transferrin

The mutual interaction between transferrin, low mol wt chelating agents and Fe(III) provides a sad example of a lack of communication between the chemical and physiological disciplines of researchers concerned with iron metabolism. Time and again statements appear in the literature to the effect that "transferrin-bound iron is not vulnerable to chelate binding" [77, 45, 78-81]. While this may be essentially valid in the context being used, unqualified assertions like this create an incorrect impression, namely that transferrin iron is always completely inaccessible to chelating agents because an equilibrium cannot be set up. There is a real danger that such a misconception can mask phenomena vital to the unraveling of iron metabolism, particularly with regard to its regulation.

The fact that iron can be removed from transferrin directly by chelating agents is well established, i.e., the reaction is reversible [82]. When serum is dialyzed against water, the amount of radio iron removed from transferrin is neglible; however, dialysis against EDTA, citrate, or NTA is effective [5, 15]. Similarly, equilibrations in which iron was dialyzed out of transferrin were featured among the

experiments used to estimate the strength of metal binding to the protein [8]. Reversible titration curves are obtained in the presence of citrate both with transferrin [8] and the similar iron-binding protein found in egg white, conalbumin [83].

The impression that transferrin iron is not available for binding to chelating agents originates from two factors. The first is the very high iron association constant exhibited by this protein. The extent to which chelating agents are able to compete with macromolecules in vitro and in vivo cannot be determined simply by comparing equilibrium constants; generally this kind of calculation can only be achieved satisfactorily by means of computer simulation (see Sec. 4). It transpires that very few low mol wt chelates at equimolar concentration with transferrin can hold a significant fraction of the total iron at equilibrium. So, the approximation that no iron is removed on these occasions is quantitatively, if not conceptually, justified. The second factor is kinetic in origin. Even with those chelating agents which, according to thermodynamic calculations, spontaneously extract measurable amounts of iron from transferrin, it may be that no such release is observed because equilibrium is not actually attained. This accounts for the many observations that chelating agents added as iron complexes are able to retain the metal in the presence of iron-binding apoprotein but are unable to strip iron from the iron-protein species.

The iron-exchange reactions between transferrin and chelating agents as well as from Fe(III) complexes to apotransferrin have been studied in detail by Bates et al. [14, 15]. The rate at which iron is transferred depends on the chemical nature and stereochemistry of the complexing agent; equilibrium concentrations are related to the magnitude of the relative stability constants. In the removal of iron from transferrin, citrate and NTA reach equilibrium at approximately the same rate (the reaction was essentially complete after 3 hr) but EDTA takes much longer. The time required to half-saturate apotransferrin binding sites was 3 sec for NTA, 8 hr for citrate, and 4 days for EDTA. The rate of donation from iron(III) citrate is

limited by the depolymerization of this system, the low mol wt product (in all likelihood the monomer) interacting rapidly [12, 14].

The transfer of iron between transferrin and chelating agents almost certainly takes place via the formation of a ternary complex [15]. The formation of a ternary species accounts for the importance of ligand stereochemistry. It also does away with the need for spontaneous separation of a Fe(III) ion which, it has been estimated [84], would take about 10,000 years!

The role of low mol wt complexes in iron sequestration by apotransferrin has been emphasised by findings that interaction with Fe(III) salts is nonspecific [2, 85, 86]. Although divalent iron is only very weakly bound to transferrin [87], at neutral pH the apoprotein reacts far more satisfactorily with Fe^{2+} than Fe^{3+}. No doubt this is due both to the hydrolysis of the Fe(III) ion and to the facilitated oxidation of Fe(II) ion in the presence of powerful iron(III) complexing agents [88]. Poor results are also found when the Fe(III) ion is generated in situ [2]. In contrast, iron from monomeric Fe(III) chelates is rapidly and stoichiometrically sequestered [2]. For example, titration of apotransferrin with Fe(III)-NTA yields a linear function having a sharp endpoint, but with iron(III) chloride only a sigmoidal curve can be obtained [86]. It has relatively recently become appreciated that these facts are most pertinent in the preparation of experimental transferrin solutions [86, 89].

Of great relevance to the question of iron transfer between physiological iron-binding sites via complexes formed by naturally occurring low mol wt ligands has been the demonstration by two separate investigations of a citrate-mediated exchange of Fe(III) ions between transferrin molecules [84, 90]. The statement that there is no appreciable movement of iron between transferrin molecules [91] again applies in the context of gross ferrokinetics only. In fact, even then a small "reflux" is noticeable which can be attributed to intermolecular transferrin iron exchange [84]. Whether this exchange in plasma has physiological importance in itself is debatable. What cannot be disputed, however, is that it provides a good model for

the phenomenon of transfer mediated by concentrations of low mol wt complexes at the limit of analytical detection [84, 90]. The implications of this, in plasma or in other biofluids, are still largely speculative, but possible effects arising therefrom should be borne in mind in both the design and interpretation of the results of future experiments.

Aisen and Leibman [84] used chromatographic differences between asialotransferrin and the native protein to monitor the iron exchange. One of these was labeled with radio iron and then incubated with an equimolar concentration of the other in the presence and in the absence of citrate. Exchange caused the appearance of two radioactive peaks corresponding to the separation of the two forms of protein. Only one radiopeak was observed when citrate mediator was absent. The exchange took 5 days to complete if the citrate concentration was 10^{-5} mol dm^{-3}, but only 24 hr if the concentration was raised to 10^{-3} mol dm^{-3}.

Ganzoni et al. [90] used the iron-donating ability of transferrin to reticulocytes to follow the ability of citrate to change diferrictransferrin into the monoferric species. (Transferrin molecules with two iron atoms are better at donating iron to reticulocytes than those with only one.) They prepared two solutions: in the first, apo- and diferrictransferrin predominated; in the second, monoferrictransferrin was formed. The solutions were preincubated with citrate for differing periods before being exposed to the reticulocytes. When the time of this preincubation was short, the first solution donated by far the greater percentage of radio iron to the cells reflecting the presence of the diferric species. With longer preincubation with citrate this difference in iron-donating ability between the two solutions disappeared commensurate with the increasing formation in the first solution of the monoferrictransferrin. The half-life of the exchange appears to be about 18 hr. Subsequently these results have been confirmed and extended [92].

3.2.2. Ferritin

Iron homeostasis is achieved primarily by manipulating the amount of this element held in the reticuloendothelial and parenchymal cells of the body by the storage proteins ferritin and hemosiderin; control by excretion is negligible, while absorption is directly related to the status of body iron stores [91, 93]. Hence, the mechanisms by which these proteins (1) remove iron when the system is faced with possible overload and (2) release it in time of need is of great interest and physiological importance. Although the importance of hemosiderin increases in siderosis, ferritin normally accounts for most of the iron in storage [93]. It also appears to be the more labile form. For these reasons most research has concentrated on ferritin, and here we shall be solely concerned with it.

Iron can be released from ferritin simply by the presence of low mol wt chelating agents. DFO liberates iron from ferritin in vivo to the limit of the chelating agent's binding capacity [79]. Removal is also achieved by 1,10-phenanthroline [94]. As the rate of release of iron in this case is independent of the chelating agent concentration, it has been reasoned that the rate-controlling step lies in the degradation of the phosphate micelles; subsequent uptake of the metal held at the ferritin surface is relatively fast [94]. Pape et al. found that physiologically significant quantities could be mobilized by specific low mol wt agents under mild temperatures and at neutral pH values [17]. NTA was much more effective than EDTA in spite of an iron-binding stability constant smaller by a factor of 10^{-9}. This led them to conclude that the avidity of the chelator for iron does not uniquely determine the rate or amount of metal mobilized [17]. Citrate was less effective than EDTA; removal of the iron depended on the concentration of this chelating agent.

Extraction of ferritin iron is most often achieved in vitro by reducing agents. Originally, sodium dithionate in the presence of bipyridyl was used, but now thioglycollic acid, an agent which can both reduce and chelate, is employed instead [93]. Although ascorbic acid, cysteine, reduced glutathione, and some other metabolites can

mobilize iron from ferritin in this manner, it does not occur at physiologically significant rates unless unrealistic concentrations of the reagents are used [95].

There is disagreement concerning the role of ascorbic acid in iron metabolism. It has long been believed that the vitamin is necessary for incorporation of iron into ferritin and therefore increases iron uptake by cells [96]. Ascorbic acid has also been implicated in iron release from ferritin both in vitro (vide supra) and in vivo [97, 98], but not all investigators agree with this conclusion [99, 100].

It is well known that apoferritin is not analogous to iron-binding proteins such as transferrin in that it does not occur in substantial quantities regardless of its degree of utilization. Rather, the presence of iron stimulates de novo synthesis and iron release is followed by protein degradation [101]. The mechanism of induction remains unclear and there is controversy about the method of iron incorporation. Historically, it has been believed that divalent iron must be present because many experiments showed that Fe(III) ions were not taken up [93]. The extent to which this tenet has been founded upon faulty results because of unsuspected Fe(III) hydrolysis reactions needs to be explored. Pape et al. found that in the presence of suitable low mol wt chelates which permit a limited polymerization, iron micelles formed that could be coated by apoferritin protein subunits thereby being transformed into ferritin [16]. Further, they noted that the product of attempts to reconstitute this protein using Fe(II) was "distinctly different" from the native substance. Their idea does suggest how ferritin induction might be prompted in vivo only in the event of increasing iron accumulation. In spite of this, Jacobs has recently concluded that primary formation of an apoferritin shell followed by iron uptake through spaces between the subunits is currently the widely accepted mechanism [101]. Whichever hypothesis is closest to the truth, one thing is absolutely clear: low mol wt Fe(III) or Fe(II) species are involved.

The movement of iron from transferrin to ferritin and back again is the process with which most of the above-mentioned arguments are ultimately concerned. The iron resides in both protein bound forms in the Fe(III) state. So the question is whether mediation by low mol wt Fe(III) complexes suffices. There is no dispute that reduction is sufficient to effect the release of the very powerfully bound transferrin iron atoms. Indeed, this has been postulated to occur at the cell membrane following transferrin attachment [102]. Miller and Perkins have produced an interesting model of the transfer between transferrin and ferritin [103]. They show that in the presence of a reducing agent which can also chelate Fe(II) reversible passive transfer of iron between the two proteins occurs. On the other hand, one can envisage a balance within the cell between the ferritin and the membrane receptor sites mediated by low mol wt Fe(III) complexes. The low concentrations that need be involved would make such a role possible for a naturally occurring ligand such as citrate.

3.3. Iron in Cells

Recently, studies concerning the regulation of iron absorption have illuminated the nature of intracellular iron-binding components and their relationships to one another. In this section we review these findings with special emphasis upon the involvement of a low mol wt fraction in cellular iron metabolism.

3.3.1. Iron in Mucosal Cytoplasm

It appears almost universally agreed that a major iron-binding species in mucosal cells is ferritin [40]. In fact, all types of cell have the ability to synthesize this storage protein when the intracellular load rises [104]. The evident production of ferritin in mucosal cells following an oral dose of iron led to proposals that it was involved in controlling iron absorption; now it is

believed to play a more passive role [40]. Iron, not immediately required by the organism, is sequestered, thus protecting against absorption. The amount of iron occurring in ferritin therefore depends to a large extent upon the needs of the animal and the amount of iron which has recently entered the cell. Hence, wide variations in the percentage of iron in the mucosal pool attributable to ferritin have been reported [64, 55, 105-107].

It also appears very likely that at least one other nonhemoprotein which specifically binds iron occurs in the mucosal cell. A substantial number of investigators have detected a high mol wt cytoplasmic carrier, apparently associated with the rapid phase of iron absorption. It is not certain whether these all refer to the same substance but consensus is for a transferrin-like protein [40]. There are good grounds for believing that this carrier determines, at least in part, the rate and amount of iron appearing at the serosal surface of the cell [40]. The evidence includes the fact that the genetic defect of mice with sex-linked anemia (s.l.a.) is associated with reduced iron binding by this transferrin-like protein [40]. However, it should be mentioned that several researches into the subcellular distribution of iron during absorption did not reveal this high mol wt species.

Halliday and Powell applied a chromatographic analysis to the supernatant liquid from intestinal cell homogenates and found four distinct iron-containing fractions [96]. Three of these were of high molecular weight; ferritin was identified and probably the transferrin-like protein described in the previous paragraph as well. The fourth low mol wt fraction was found consistently. The three fractions, other than ferritin, appeared within 2 min of radio iron administration which suggests they are involved in the transport of the metal across the cell as well as into ferritin. On the other hand, electrophoretic studies by Linder et al. [64] indicated that the iron taken up by the mucosal cell is about equally distributed between ferritin and the low mol wt fraction.

Some of these studies refer to the possibility of free ionic iron in the cytoplasm [64, 96, 107]. In view of what has already been said in the introduction, significant proportions of Fe(III) aquo-ion are out of the question and even Fe(II) is probably to be found associated with low mol wt ligands present in the biofluid. So, in the authors' opinion, the observations more likely constitute evidence for a low mol wt complex fraction. The identity of these complexes will be discussed in Sec. 4. However, it is interesting to note that the labile nature of this pool can easily be responsible for reports of a confusing nature if they are not interpreted in this light. Brown and Rother identified a low mol wt fraction and then, using chromatography, decided that the ligands involved were glycine and serine [105, 106]. Several investigators could not confirm that it was these amino acids that were complexed to the iron. Instead, they attributed the earlier observations to the use of EDTA in the preparative procedures [107-109]. Clearly, the low mol wt complex distribution will readily rearrange whenever the equilibrium is disturbed [1]. The low mol wt fraction has been made all the more elusive by its adherence to Sephadex columns [64, 100, 107].

3.3.2. A Labile Iron Pool

While work on this review has been in progress, Jacobs has published an article surveying the evidence for labile iron pools within all cells [104]. Hence, there is no need to review this subject in detail. Rather, an attempt will be made to provide a slightly different perspective.

In general, cellular iron uptake, say from membrane-bound transferrin, is diverted between two pathways: (1) to the mitochondria for metalloprotein synthesis and (2) to ferritin for storage. In some cells, such as those from the intestinal epithelium, other specific iron-binding macromolecules probably occur as intermediates although the evidence presently favors the view that "transferrin-like" proteins do not exist in all cases [104]. It is therefore pertinent to try to identify the intracellular agents which trans-

port iron between the various known binding sites and to elucidate their properties and contribution to iron metabolism.

The evidence in favor of low mol wt complex participation in the labile iron pool is substantial [100, 110-115]. It also appears that an equilibrium is set up between this low mol wt fraction and (1) transferrin or chelating agents outside the cell [100, 104] and (2) ferritin within the cell [116]. Enlargement of the "prerelease" iron pool in reticuloendothelial cells probably causes the induction of ferritin synthesis [116]. There may even be an equilibrium set up between Fe(II) and Fe(III) compounds determined by the redox potential within the cell [104].

It is quite probable that the size of the low mol wt complex pool is the cause of most disagreement over the labile iron content of cells. It seems highly unlikely that the low mol wt Fe(III) fraction will be quantitatively significant for two reasons: (1) the ever-present competition from nonspecific protein binding sites which occur in relatively high effective concentration and (2) the general impression which can be obtained from computer simulations (see Sec. 4). This is not as true for Fe(II) even if only because of its greater solubility. In most biofluids, the size of the labile pool will not be the same as the size of the low mol wt complex fraction; reports that 95% of the cytosol radio iron is chelatable by DFO or even that 35% of the cytosol nonheme, nonferritin iron is dialyzable [100] do not refer to the amount of low mol wt complexed iron; they merely indicate that this quantity of iron, in kinetic terms, is rapidly released to low mol wt chelating agents.

3.4. Chelation Therapy

There are three important pathologies in which iron overload causes massive deposition of the metal in the parenchyma of a number of organs, causing tissue damage and ultimately proving fatal [117].

These are *idiopathic haemochromatosis, Bantu siderosis,* and *transfusional siderosis.* In the first two cases, overload is consequent to excessive absorption and is therefore most easily treated by phlebotomy. The fact that 250 mg of iron is removed for every 500 ml of whole blood lost should be borne in mind throughout this discussion of the alternative chelation therapy. However, in the case of blood transfusions, phlebotomy is obviously not a realistic proposition. Repeated transfusions constitute the only effective treatment for sufferers of chronic aplastic anemia or β-thalasemia major [53, 114]. The large intake of iron associated with this treatment cannot be homeostatically controlled because the body has no means of significantly increasing iron excretion. So a pathological buildup of the metal occurs and most of the afflicted die in their teens from the heart or liver failure that is caused [114].

There is consequently a serious need to develop iron chelating agents which can be used to combat iron overload. Unfortunately, those in common use today are not very satisfactory; they are by no means as effective as could be hoped and oral administration is not currently possible due to poor absorption.

Urinary iron excretion is not significantly enhanced by any of the common chelating agents except in cases of overload [74]. The most commonly used therapeuticals are DFO and DTPA, both of which can achieve iron losses up to 50 mg per day [74, 79, 118]. More often 10-15 mg per day is excreted in the urine of patients receiving these two agents [119-121]. Neither EDTA nor HEDTA are as effective [34, 46, 119] although some potentially better agents have been recognized [53, 122-124]. Although differing experimental conditions make it difficult to compare these studies, it is possible to produce a list of iron-chelating agents in order of decreasing effectiveness:

DFO, EHPG > CDTA > DTPA > EDTA, HEDTA > NTA

A considerable effort has been expended researching the chemistry and physiology of desferrioxamine, DFO, and its iron chelate ferrioxamine. The ligand is a hydroxamic acid condensation product of acetic and succinic acids with L-amino-5-hydroxylaminopentane [79].

The three hydroxamic groups each lose a proton to bind Fe(III) with extraordinary avidity. The complex formed has a single positive charge at physiological pH values due to the protonation of an amino side group. In addition, the polarity of the molecule is increased by partial negative charges on the nitroso functions [48]. This explains why ferrioxamine is poorly absorbed from the gastrointestinal tract and excreted by renal filtration [79].

As a chelating agent, DFO is certainly one of the most specific; it is not found to influence the excretion of any other trace element [79]. It suffers from two disadvantages, however. It has a very short half-life in vivo, probably less than 1 hr [79]. Also, its phenomenal binding strength depends upon its ability to wrap itself around the Fe(III) ion. It is therefore unable to form a satisfactory ternary complex with transferrin. Instead it must rely either upon spontaneous dissociation (vide supra) or upon other chelating agents which can mediate in an iron exchange. Thus the reaction, although thermodynamically favored, is slow. In practice, the short metabolic half-life means that DFO is unable to obtain significant amounts of iron from transferrin in plasma. Thus, DFO has no effect on plasma ferrokinetics [77-80] and excretion of transferrin-bound radio iron is not enhanced by DFO administration [77, 80]. The occurrence of nonspecifically bound iron in plasma has probably given rise to the few conflicting reports [79, 80].

The physiological site of iron-chelating agent action has been a widely discussed topic. No clear answer has been forthcoming except that neither transferrin nor the gastrointestinal tract can be a major source [77, 81]. The effect on organ radioactivity appears limited to the liver so the problem really is whether the iron stems from parenchymal or reticuloendothelial cells. However, a fact which seems to be forgotten too often is that if the system is sufficiently labile (Sec. 3.3), the site of action and the body compartment from which most iron disappears are not necessarily one and the same.

The situation with DTPA is simpler than with DFO. This polyaminocarboxylate forms complexes in plasma that are highly charged,

so it does not penetrate intracellular space [125]. Accordingly, it binds iron in the plasma compartment once transferrin has been saturated [126]. Whether the amount of chelatable iron in plasma is at equilibrium with body stores is not clear [81, 126].

The evidence suggesting that DFO-induced iron excretion is derived from reticuloendothelial (RE) cells was put forward by Karabus and Fielding [127]. They suggested that the chelatable iron was derived from hemoglobin when mature or defective red blood cells are catabolized. Iron in the RE cell was proposed to be in equilibrium with storage iron on the one hand and transport iron on the other; DFO sequestered the metal from this pool. Their view was supported by the observations of Lipschitz et al. who thought that the major immediate source was probably an unknown compound on the pathway between ferritin and plasma transferrin [128].

However, these suggestions have been refuted by Finch and coworkers [77, 129] whose evidence indicates that DFO enters liver parenchymal cells where it binds excess storage iron.

The analysis and interpretation of the experimental data concerning DFO chelation therapy are further complicated by the fact that this agent is not completely confined to the extracellular space [79, 81]. Following DFO administration, about one-third of the induced iron excretion appears in the feces via the bile [124, 129]. However, intravenously administered ferrioxamine does not follow this pathway; it is largely unable to enter the hepatic parenchymal cells (vide supra). These facts strongly support the view that at least some of the DFO enters these liver cells and therein chelates iron [77, 129].

The conclusion that the remaining two-thirds of the intracellularly formed ferrioxamine passes back into plasma to be excreted by the kidney [77] is somewhat less sound. To begin with it assumes that ferrioxamine is capable of traversing the membrane only in one direction. No evidence in support of this contention is provided. It seems highly unlikely that iron in plasma which is available to DTPA is not likewise chelated by DFO. Until considerably more

evidence on this matter is forthcoming, it is probably wise to assume that the fraction of iron excreted via the urine is chelated in the blood plasma compartment, while that appearing in bile arises from DFO penetration of the parenchymal cell. The ability of the ligand to traverse this membrane is discussed further in Sec. 4. The manner in which the chelating agents bind iron in plasma remains speculative. The idea that they compete with transferrin at the cell membrane [127] has been disputed [81]. Nevertheless, it is known that following DFO administration, serum iron concentrations increase owing to the mobilization of iron from tissues [130].

3.5. Regulation of Iron Metabolism

Ever since it was discovered that the body has very limited ability to excrete iron either in urine or feces, it has been realized that homeostasis is maintained by regulating the rate of iron absorption [131]. This finding has prompted many hundreds of investigations aimed at elucidating how the mucosal cell senses and responds to the need for this essential trace element. The question is how do these parameters influence the mucosal cell? Explanations must lie in changes within the cell itself because absorptive characteristics are imprinted upon gut segments. The failure to demonstrate a role for (other) humoral factors makes it increasingly obvious that the homeostatic feedback is via iron itself although how this is achieved is not easily shown.

Current ideas concerning the regulation of iron metabolism are presented in Chap. 9. Here, special emphasis needs to be given to the possible role of equilibrium and low mol wt complexing phenomena.

Saltman has proposed a model of iron metabolism in which absorption is almost completely controlled by chelation, solubility, diffusion, and equilibrium [10, 23]. Solubilization of the iron in the lumen by low mol wt chelates is followed by their penetration into the mucosal cell. Subsequently, their passage is controlled

only by their interaction with binding sites in the cell, such as those associated with ferritin, other proteins, or other low mol wt ligands. The iron is released directly into the blood stream as a low mol wt Fe(III) chelate which there reacts with transferrin. There can be little doubt that with lipophilic complexes strong enough or inert enough to survive the competition for iron within the mucosal cell, this hypothesis adequately describes the position. However, no control is exerted under these circumstances. The extent to which it also applies when only weaker complexes could be absorbed is of great interest.

Recently Cavill et al. have suggested that iron absorption is regulated by internal iron-exchange considerations, namely by the relative sizes of exchangeable iron pools within the body and by the rate at which iron is cleared from the plasma by erythropoeisis [132]. They argue that the probability of an iron atom being picked up from the intestinal lining by a transferrin molecule in plasma is proportional to the ratio of exchangeable iron in that tissue to the exchangeable iron in the whole body. The number of iron atoms transferred from all body compartments into plasma is equal to the number cleared, so absorption is related to the expression,

$$\frac{\text{Intestinal exchangeable iron}}{\text{Total exchangeable iron}} \times \text{plasma iron turnover}$$

This explains how iron absorption may be altered according to either iron status or erythropoietic activity. Cavill et al. cite several other instances where this relationship accounts satisfactorily for experimentally observed phenomena in iron metabolism [132]. Their proposal implies that serosal transfer of iron is governed by an equilibrium between plasma iron and the exchangeable iron in all tissues [104].

If an equilibrium between exchangeable iron pools does indeed regulate iron metabolism it seems more than likely that mediation by low mol wt complexes will be important. This will be considered further in Secs. 4 and 5.

Cavill et al. do not suggest the means through which the equilibrium is maintained, they only consider the one-way reaction from mucosal cells into plasma. However, if any control is to be exerted, iron must also be returned. Two possibilities which need investigation are clear: Is transferrin able to donate sufficient iron to the mucosal cell once it has matured, or is the feedback achieved only while the cell is being formed?

A further difficulty is to be found when one considers experimental data concerning transferrin and iron absorption. There is conflicting evidence on whether transferrin concentration or saturation regulates the transfer of iron into plasma. On the whole it appears that it does not, in which case the longstanding issue of how the mucosal cell is informed of the need for iron remains unresolved. Solvell found that while the total amount absorbed was unaffected by transferrin saturation, the rate of transfer was influenced markedly [133]. So it looks as if the kinetics of this complicated system may obscure exact relationships (Sec. 5). Further, it is tempting to speculate that the functional heterogeneity of transferrin as proposed by Fletcher and Huehns [134] may prove to be the key to understanding this problem: iron absorption may be related primarily to the concentration and saturation of the reticulocyte-oriented iron-binding site.

4. COMPUTER SIMULATION STUDIES

The investigation of low mol wt complexes in biofluids by experimental techniques is virtually excluded by the extremely low concentrations involved and by the labile nature of the equilibria. In principle, some insight can be obtained by applying knowledge gained from relatively large-scale experiments in vitro, but if such attempts are to be anything more than qualitative guesses, it is essential to take all the relevant factors operating on the multicomponent system into account. These include the concentrations of the components as well

as the equilibrium constants of all the competitive binding interactions for the metal ions in the biofluid. Powerful computer programs are available to accomplish this task; models of 5,000 potentially important complex species formed by 40 ligands are presently possible [1]. Recently it has been shown that despite a lack of quantitative information about metal-protein binding in the biofluid, computer simulations are able to yield a number of useful conclusions concerning the low mol wt fraction. For example, the percentage distribution of transition metal ions in normal plasma can be obtained [1]. Further, the models show that physiological interactions between transition metal ions do not reflect competitive effects on the low mol wt equilibria in blood plasma.

Naturally, the difficulties associated with experimental studies of Fe(III) complexes in aqueous solution have severely affected computer models. The importance of iron as a trace metal is simply not consistent with the number of Fe(III) ion formation constants available from the literature or with the reliability of many of those reported. Nevertheless, certain conclusions pertinent to iron can be drawn from those computer studies so far conducted.

1. The models show that at neutral pH, citrate enjoys a considerable advantage over the other low mol wt (Fe(III) ion-binding agents occurring in plasma. In all likelihood, ternary Fe(III) complexes will predominate with citrate being at least one of the ligands. Until more formation constant data become available the other ligands cannot be identified with as much certainty. Salicylate^{2-}, oxalate^{2-}, and glutamate^{2-} are strong possibilities but hydroxide anion presently seems the most likely. Even in this event, several species have been postulated (see Sec. 2); our current models suggest Fe•citrate•OH^- and Fe•citrate•OH_2^{2-} in approximately equal concentrations. Spectrophotometrically determined formation constants for Fe(III) hydroxyphosphate complexes [135] indicate these should also be considered; quantitatively, this is not yet feasible because (1) the free phosphate ion concentration in plasma is still ill defined [1] and (2) the above-mentioned study appears to have neglected the well-known binuclear $Fe_2(OH)_2$ species (Sec. 2).

2. In spite of the many uncertainties listed in item 1, the evidence of the models strongly supports the idea that the low mol wt Fe(III) complexes which predominate in plasma are all negatively charged. This is a consequence of the ubiquitous citrate complexing in conjunction with any other anionic ligand.

3. At least 10 orders of magnitude separate the concentrations of the predominant low mol wt complexes and that of the aqueous Fe(III) ion. This figure pertains to the Fe·citrate·OH^- and Fe·citrate·OH_2^{2-} species mentioned in item 1 which are estimated to exist in concentrations of about 10^{-12} mol dm^{-3}. Of course, these figures would be increased if it transpired that another complex was more important.

As only very small amounts of hemoglobin and ferritin occur in blood plasma almost all the Fe(III) in this biofluid therefore is bound to transferrin. Indeed, it is probable that less than 0.000001% of the iron in plasma is complexed to low mol wt ligands. Two reasons why the concentrations of low mol wt Fe(III) complexes need to be repressed, in addition to those applicable to transition metals generally, are reflected in the extraordinary iron-transferrin binding strength: (1) bacterial proliferation depends upon iron bioavailability, and (2) loss by renal excretion is effectively avoided thus satisfying the paramount need to conserve this trace element.

Some experimental attempts to identify the low mol wt complexes in plasma have been made by measuring the amount of low mol wt iron in native and predialyzed serums incubated with various amounts of added radioactive metal [136, 137]. They show a dramatic decrease in the percentage of radio iron in the ultrafiltrate/supernatant when low mol wt ligands are absent. However, these studies do not necessarily identify the ligands normally involved because the pertinent effect is only exhibited in the presence of unphysiological amounts of the metal (i.e., after the specific binding sites of the protein have been saturated). This exemplifies the hazards of drawing conclusions from experiments in which the equilibrium is severely disrupted. It may also account for the different results obtained [136, 137].

Perhaps the most promising application of computer models lies in their ability to predict how well administered agents can bind metal ions in vivo [125]. Almost all drugs are potential metal-binding agents but far fewer can compete successfully with endogenous ligands. Clearly, some identification of those which can do so is desirable as is a knowledge of the relative avidities they display towards different kinds of metal ion. Comparison of two or more systems simply on the basis of the 1:1 complex-formation constant is a common malpractice: such neglect of the competitive effects of other metal ions and especially of protons often leads to palpably nonmeaningful results.

The introduction of a plasma mobilizing index (PMI) calculated from the computer simulation results makes a valid comparison among the chelating agents in blood plasma possible [125]. The index is defined as follows:

$$\text{PMI} = \frac{\text{total concentration of low mol wt metal complex species in the presence of drug}}{\text{total concentration of low mol wt metal complex species in normal plasma}}$$

By formulating the index in such a manner, it is independent of the exact free-metal ion concentration chosen for the simulations, so errors arising from the uncertainty associated with these values due to poorly categorized metal-protein equilibria are avoided.

PMI curves for a number of chelating agents with ferric ion are depicted in Fig. 1 (Table 1). There is good correlation between the order of the curves and the decreasing effectiveness of these compounds as iron-removing therapeuticals listed in Sec. 3.4. The results show that several agents are able to compete effectively with transferrin for Fe(III) ion. The strongest include DFO and EHPG. Reasons why they may be unable to remove Fe(III) ions already complexed to this iron-binding protein have been discussed (see Sec. 3.4).

A model of blood plasma in which the exchange of iron between DFO and transferrin is restricted showed that neutral, doubly

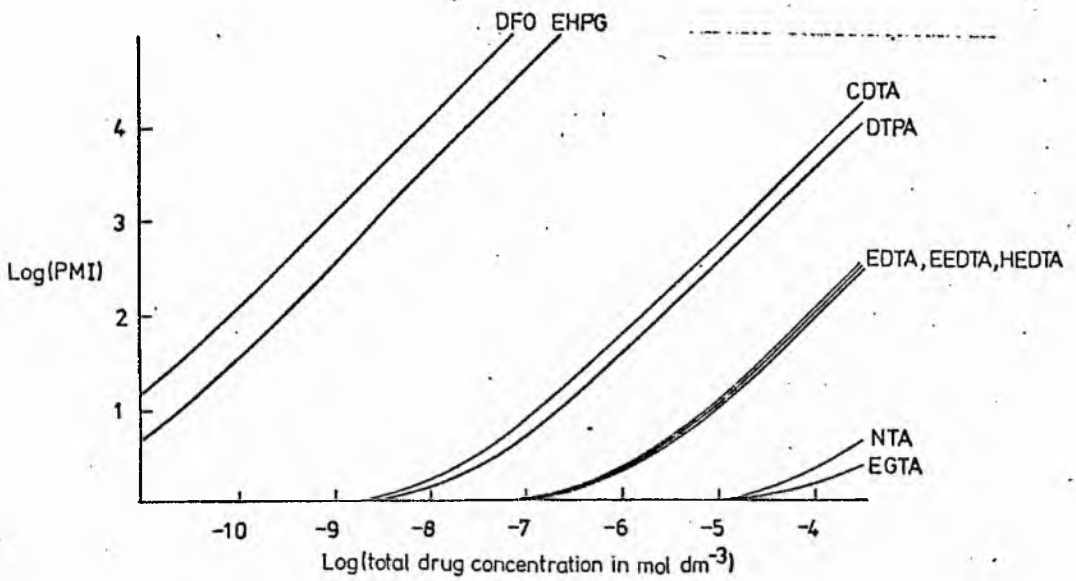

FIG. 1. PMI curves calculated for Fe(III) chelating agents by using computer simulation and the formation constants given in Table 1.

protonated DFO accounts for about 10% of the ligand. The intracellular penetration of this chelating agent may thus be attributed to this, the most lipophilic DFO species predominating in the biofluid.

At present, models for low mol wt complexation in biofluids other than plasma have not been constructed or suffer from certain theoretical limitations. For example, the nature of the metal ion binding to low mol wt ligands in cytoplasm awaits elucidation. However, if such fluids are less oxidizing than plasma, there is the possibility that low mol wt Fe(II) complexes may exist. The ligands which are most likely to be found complexed to Fe(II) may be suggested by inserting Fe(II) into the plasma model. (This tacitly assumes that the ligand and metal ion composition and especially the pH of the biofluid are not too unlike that of plasma.) The suggested percentage distribution of Fe(II) complexes is shown in Table 2. This indicates that the carbonate and ascorbate complexes are likely to be amongst the most predominant low mol wt Fe(II) species in vivo at about neutral pH. While this inference is by no means rigorous, it is

TABLE 1

Important Species Formed by Chelating Agents in Blood Plasma as Found by Computer Simulation

Chelating agent administered[a]	Species formed	Formation constant (log values)[b]	Percentage total ligand[c]	Percentage total Fe(III) in low mol wt fraction[c]
DFO	$Fe{\cdot}DFO^{+}$	29.8	98	99
	$Fe{\cdot}DFO{\cdot}OH$	34.0	1	1
EHPG	$Fe{\cdot}EHPG^{-}$	33.5	98	100
CDTA	$Zn{\cdot}CDTA^{2-}$	18.6	77	
	$Ca{\cdot}CDTA^{2-}$	12.0	22	
	$Fe{\cdot}CDTA{\cdot}OH^{2-}$	35.0	~0	86
	$Fe{\cdot}CDTA^{-}$	28.0	~0	14
DTPA	$Zn{\cdot}DTPA^{3-}$	17.8	91	
	$Ca{\cdot}DTPA^{3-}$	10.6	7	
	$Fe{\cdot}DTPA^{2-}$	27.8	~0	99
EDTA	$Ca{\cdot}EDTA^{2-}$	10.4	73	
	$Zn{\cdot}EDTA^{2-}$	16.0	26	
	$Fe{\cdot}EDTA^{-}$	24.8	~0	51
	$Fe{\cdot}EDTA{\cdot}OH^{2-}$	31.0	~0	48
HEDTA	$Zn{\cdot}HEDTA^{-}$	14.2	58	
	$Ca{\cdot}HEDTA^{-}$	8.0	41	
	$Fe{\cdot}HEDTA{\cdot}OH^{-}$	29.1	~0	97
EEDTA	$Ca{\cdot}EEDTA^{2-}$	9.8	91	
	$Zn{\cdot}EEDTA^{2-}$	14.8	8	
	$Fe{\cdot}EEDTA^{-}$	24.2	~0	72
	$Fe{\cdot}EEDTA{\cdot}OH^{2-}$	30.0	~0	27
NTA	$Ca{\cdot}NTA^{-}$	6.2	88	
	$H{\cdot}NTA^{2-}$	9.5	6	
	$Fe{\cdot}NTA{\cdot}OH^{-}$	24.6	~0	31
EGTA	$Ca{\cdot}EGTA^{2-}$	10.3	100	
	$Fe{\cdot}EGTA{\cdot}OH^{2-}$	28.5	~0	12

[a] Symbols represent anionic forms of the agents defined in Sec. 6; in addition, Fe(III), Ca(II), and Zn(II).

[b] Formation constants have been selected from the literature and corrected to conform with the temperature and ionic strength of blood plasma as previously described [1, 125].

[c] Percentages refer to a total ligand concentration = 10^{-4} mol dm^{-3}.

TABLE 2

Percentage Distribution of Fe(II) Ions Among Low Molecular Weight Human Blood Plasma as Found by Computer Simulation (-log $[H^+]$ = 7.4)

Complex	Charge	Percentage of the total metal in the low mol wt fraction
Protonated carbonate	1+	27
Carbonate	0	21
Ascorbate	0	13
Histidinate	1+	8
Citrate	1-	5

noteworthy that if these neutral complexes are significant in cytoplasm, they could be responsible for a unidirectional passive flux into plasma. Thus, they would be the compounds suggested by Lipschitz et al. as the major immediate donors of iron to DFO (Sec. 3.4). Such speculation is to some extent supported by the iron excretion induced by the Fe(II) chelating agents bipyridyl and 1,10-phenanthroline [53].

Computer simulations, like all models, are often regarded with deep suspicion. This is a misplaced distrust, better reserved for careless interpretation of results of the models. Models are an integral part of the scientific method: they are constructions attempting to imitate by representation. As such, they exhibit the strengths and weaknesses in the understanding of the system in question, not only serving to expose fallacious hypotheses but also being an extremely useful aid in the interpretation of experimental results which are consistent with current ideas.

5. CONCLUSIONS

Reference has been made throughout this chapter to the difficulties associated with studies of Fe(III) complexing equilibria. Our motive has been to emphasize the extent to which the understanding of iron

metabolism is thereby restricted. While any study of biological ligand-biometal interactions is of interest, those pertaining to their behavior in their biological environment are indisputably of greatest value. The physiology of a trace element is primarily a reflection of how the concentrations of its compounds are regulated in vivo. Thus, in spite of the problems inherent in studies of biological concentrations, it is important that research efforts do not polarize away from those techniques which illuminate the kinetics and equilibria of iron species in solutions under physiological conditions.

The distinct advantages of computer models to such studies have been outlined in Sec. 4. There is an obvious need to extend such simulations to embrace both the kinetically controlled distribution of metal ions between body compartments as well as equilibrium constraints imposed in each of the respective fluids. Although much of the necessary computer input data is not yet available, in our opinion, a viable model could nevertheless be constructed. The primary objective would be to faithfully represent the observed ferrokinetic parameters and their dependence upon prevailing conditions.

The protracted quest to uncover the mechanism controlling iron absorption has properly been directed at finding and characterizing the rate-determining step. This has meant that most experiments have studied a single process. However, it now seems probable that all the factors important in influencing iron absorption are not mediated through only one process. Overall control, therefore, resides in the complicated interplay of individual reactions. For example, the increase in iron absorption that occurs in patients deficient in iron is not merely a reversal of the decreased absorption occurring in iron overload [138].

Although present models provide much information concerning the role of low mol wt Fe(III) complexes, a more sophisticated simulation that also depicts the relationship between body compartments would be particularly useful. It could reflect the extent to which low mol wt

complexes need to be invoked if equilibrium is indeed the key to the regulation of iron metabolism. Clearly, the transfer of low mol wt complexes through biological membranes depends upon many factors; the concentrations of species on either side of the membrane, their molecular weights, and lipophilicities all contribute to the rates at which equilibrium is approached. The situation is further complicated by the ability of transferrin to become attached to some varieties of cell while accepting or donating metal ions. This may bypass the necessity for low mol wt Fe(III) complexes to transport the metal through membranes (e.g., the serosal membrane of intestinal epithelium) under normal circumstances; an evolutionary adaptation (it is tempting to speculate) necessitated by problems arising from a predominance of negatively charged low mol wt species (Sec. 4). Under these circumstances, low mol wt complexes would be confined to mediating the exchange of metal between macromolecular iron-binding components in the cell and the transferrin receptors attached to the membrane.

The value of correlating computer simulation and biological response data ought to be emphasized. While the participation of low mol wt complexes in iron physiology does not necessarily require high in vivo concentrations, the nature and concentration of these complexes will in part determine the behavior of the system in which they occur. Thus it is in these observable phenomena that the role of low mol wt iron complexes is manifest and from which their biological significance must be deduced.

ABBREVIATIONS

ATP: adenosine 5'-triphosphate
CDTA: cyclohexane-1,2-diaminetetraacetic acid
DFO: desferrioxamine
DTPA: diethylenetriaminepentaacetic acid
EEDTA: bis-2-aminoethylethertetraacetic acid
EDTA: ethylenediaminetetraacetic acid

EGTA: ethylenebis(oxyethylenenitrilo)tetraacetic acid

EHPG: ethylenediaminebis(o-hydroxyphenyl)glycine

HEDTA: hydroxyethylethylenediaminetetraacetic acid

NTA: nitrilotriacetic acid

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